

## **Supplementary Information**

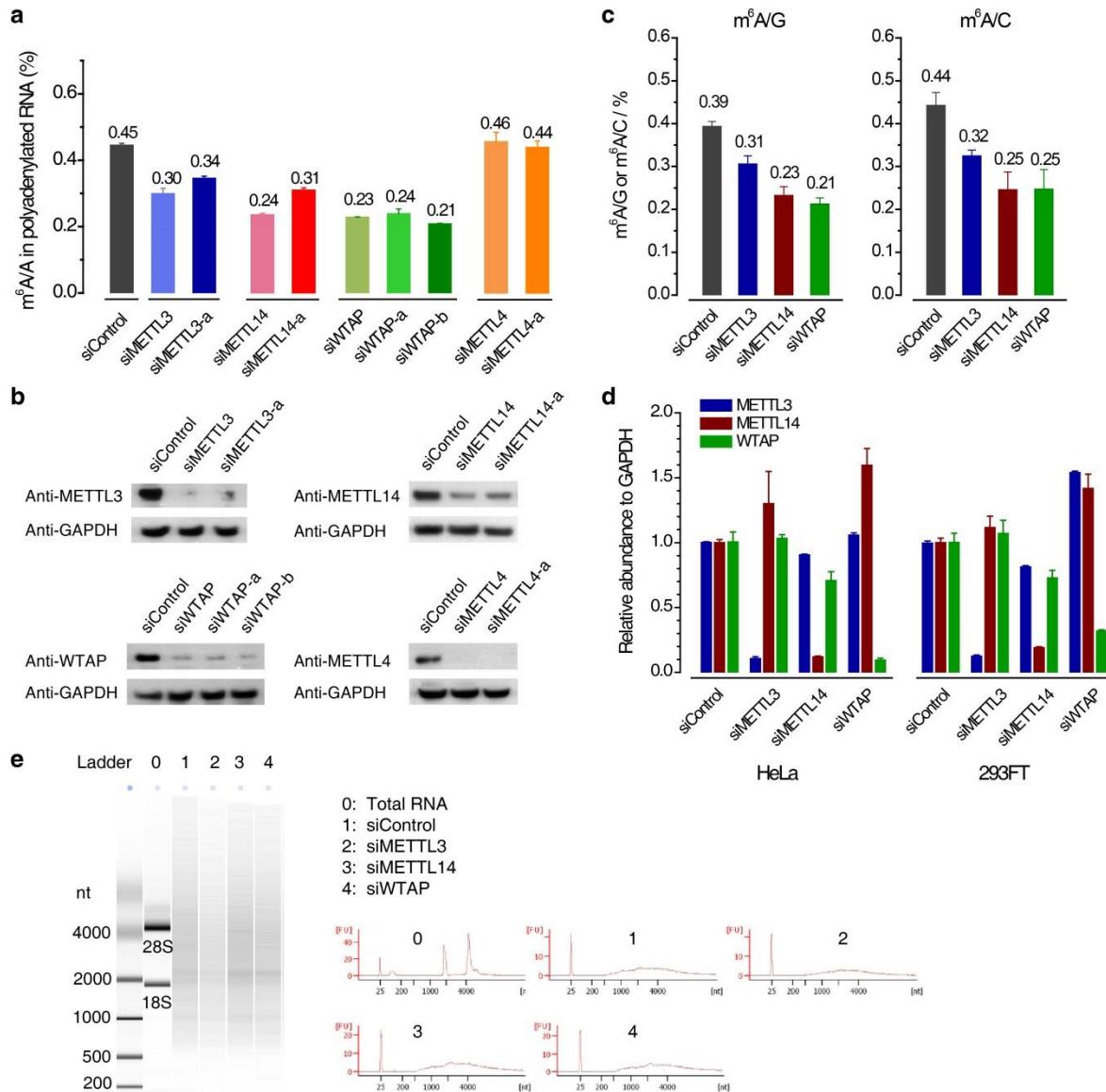
### **A METTL3-METTL14 complex mediates mammalian nuclear RNA *N*<sup>6</sup>-adenosine methylation**

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2. These authors contributed equally to this work

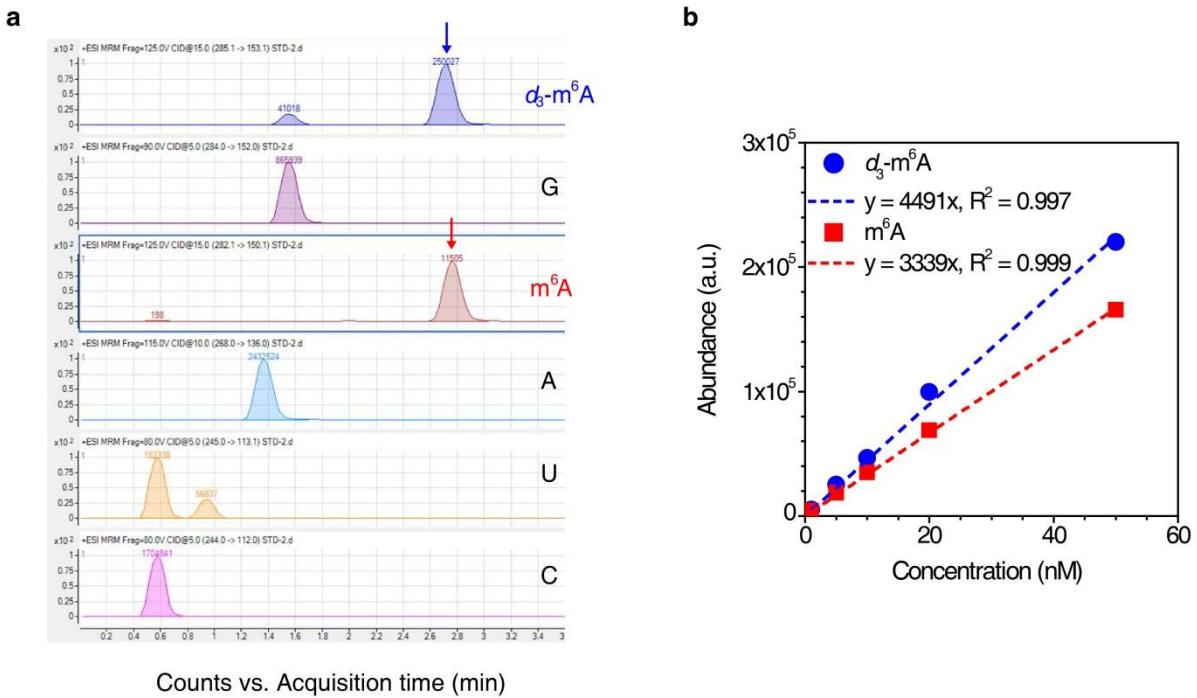
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## Supplementary Results

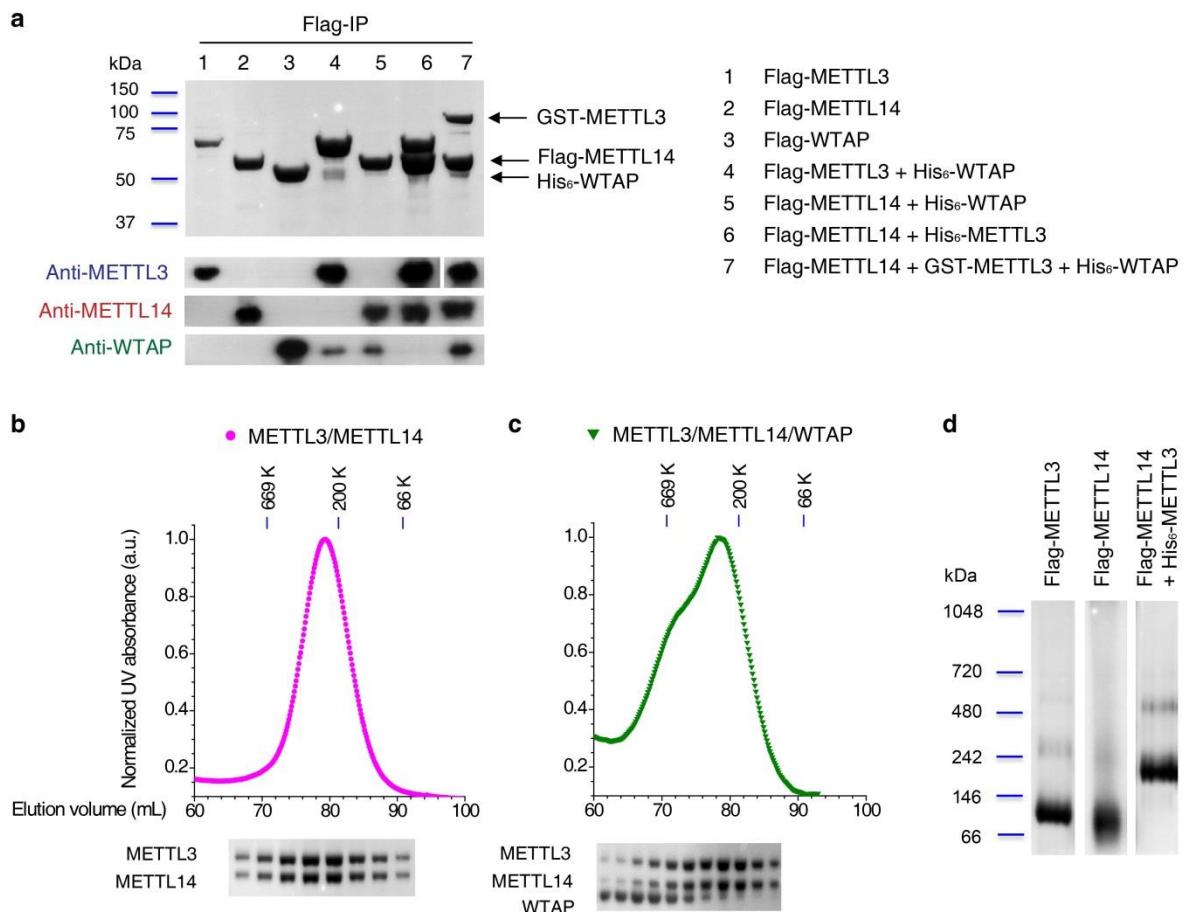


**Supplementary Figure 1 | METTL3, METTL14, and WTAP regulate m<sup>6</sup>A content in polyadenylated RNA inside cells.** (a) Quantification of the m<sup>6</sup>A/A ratio in polyadenylated RNA by LC-MS/MS, where different siRNAs for each target gene were applied to confirm the effect of knockdown on the cellular m<sup>6</sup>A level. A significant decrease in m<sup>6</sup>A content was observed in *METTL3*, *METTL14*, and *WTAP* siRNA-treated HeLa cells after 48 h ( $P < 0.001$ , Student's *t*-test, calculated between control and specific knockdown sample). In contrast, knockdown of *METTL4* gave no obvious change in the m<sup>6</sup>A level in polyadenylated RNA ( $P > 0.53$ , Student's *t*-test). (b) Western blotting of all the samples shown in (a). The siMETTL3, siMETTL14, and siWTAP showing an optimal knockdown efficacy were chosen for the subsequent experiments. (c) Comparison of the m<sup>6</sup>A/A ratio with m<sup>6</sup>A/G(C) ratio showing

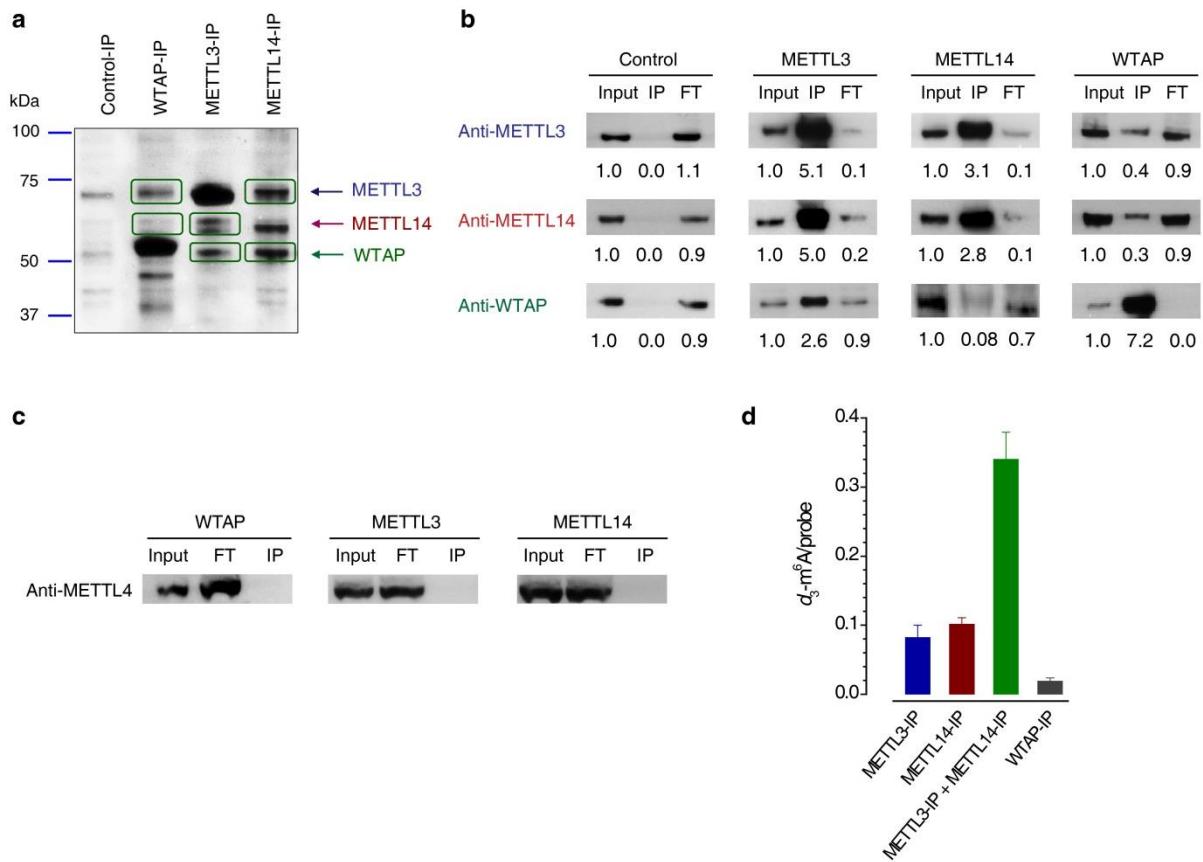
that the change of mRNA m<sup>6</sup>A methylation is not caused by the global alternation of A/C/G/U in the transcriptome. This comparison was made for all experiments with one example shown here. Error bars in (a) and (c) indicate mean  $\pm$  s.d.,  $n = 4$ . (d) Relative mRNA level of *METTL3*, *METTL14*, and *WTAP* in samples shown in **Figure 1a**, as detected by RT-qPCR. Error bars indicate mean  $\pm$  s.d.,  $n = 3$ . (e) The quality of polyadenylated RNA isolated from the HeLa samples was analyzed by the Agilent 2100 bioanalyzer, where the plots in right panel are the corresponding RNA length distribution profiles. rRNA in each sample was depleted almost to the same extent.



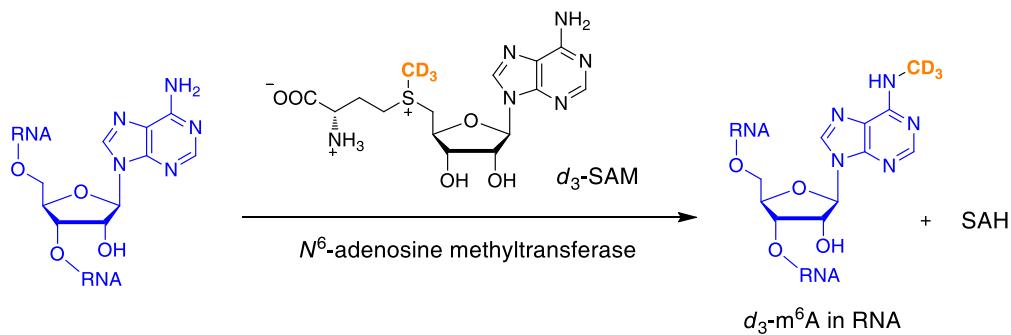
**Supplementary Figure 2 | Representative LC-MS/MS spectra and the standard curves for m<sup>6</sup>A and d<sub>3</sub>-m<sup>6</sup>A.** (a) On a LC-MS/MS system, the amount of each nucleoside was quantified by its integration area in the corresponding chromatogram. (b) The m<sup>6</sup>A and d<sub>3</sub>-m<sup>6</sup>A standard calibration curves were obtained from five standard samples with different amounts of pure m<sup>6</sup>A and d<sub>3</sub>-m<sup>6</sup>A, respectively.



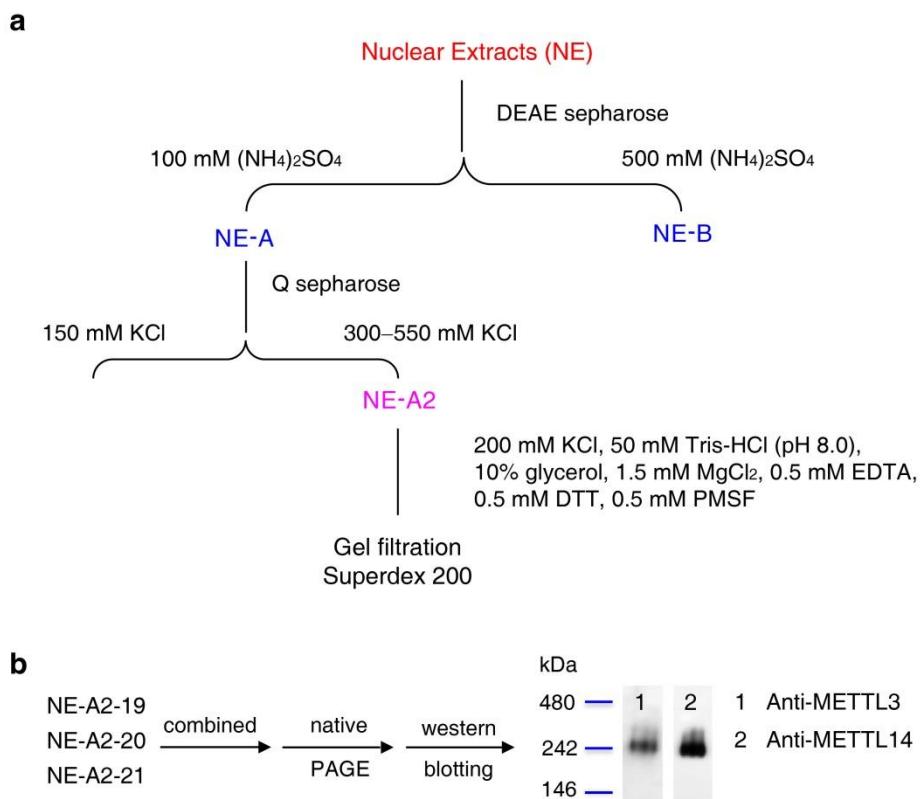
**Supplementary Figure 3 | METTL3 and METTL14 form a stable heterodimer complex *in vitro*.** (a) Proteins with different tags including Flag, GST, and His<sub>6</sub> were co-expressed in SF9 insect cells. The co-immunoprecipitation (co-IP) products pulled down by anti-Flag beads were subject to SDS PAGE (top panel, detected by Coomassie staining) and western blotting analysis (bottom panel). METTL3 and METTL14 show a strong affinity to each other as indicated by the pronounced co-IP bands for both proteins in lanes 6 and 7. A significantly lower stoichiometry of WTAP to METTL3 or METTL14 was observed in lane 4, 5 or 7. (b and c) Gel filtration trace and corresponding SDS PAGE (detected by Coomassie staining) of the Flag-IP from insect cells co-expressing Flag-METTL14/His<sub>6</sub>-METTL3 (b), as well as Flag-METTL14/Flag-METTL3/Flag-WTAP mixture with a molar ratio of 1:1:1 (c). The isolated METTL3-14 complex contains a 1/1 stoichiometry of METTL3/METTL14 as revealed by SDS PAGE in (b). The interactions between WTAP and METTL3-14 complex could be detected but are weak (note the protein stoichiometry in each fraction shown in (c)). Markers: 669 kDa (thyroglobulin, bovine), 200 kDa ( $\beta$ -amylase from sweet potato), and 66 kDa (bovine serum albumin). (d) Coomassie staining of native PAGE of the individual Flag-tagged METTL3 and METTL14, as well as the Flag-IP from insect cells co-expressing Flag-METTL14/His<sub>6</sub>-METTL3. A dimer formation was observed in the co-expression of METTL3 and METTL14.



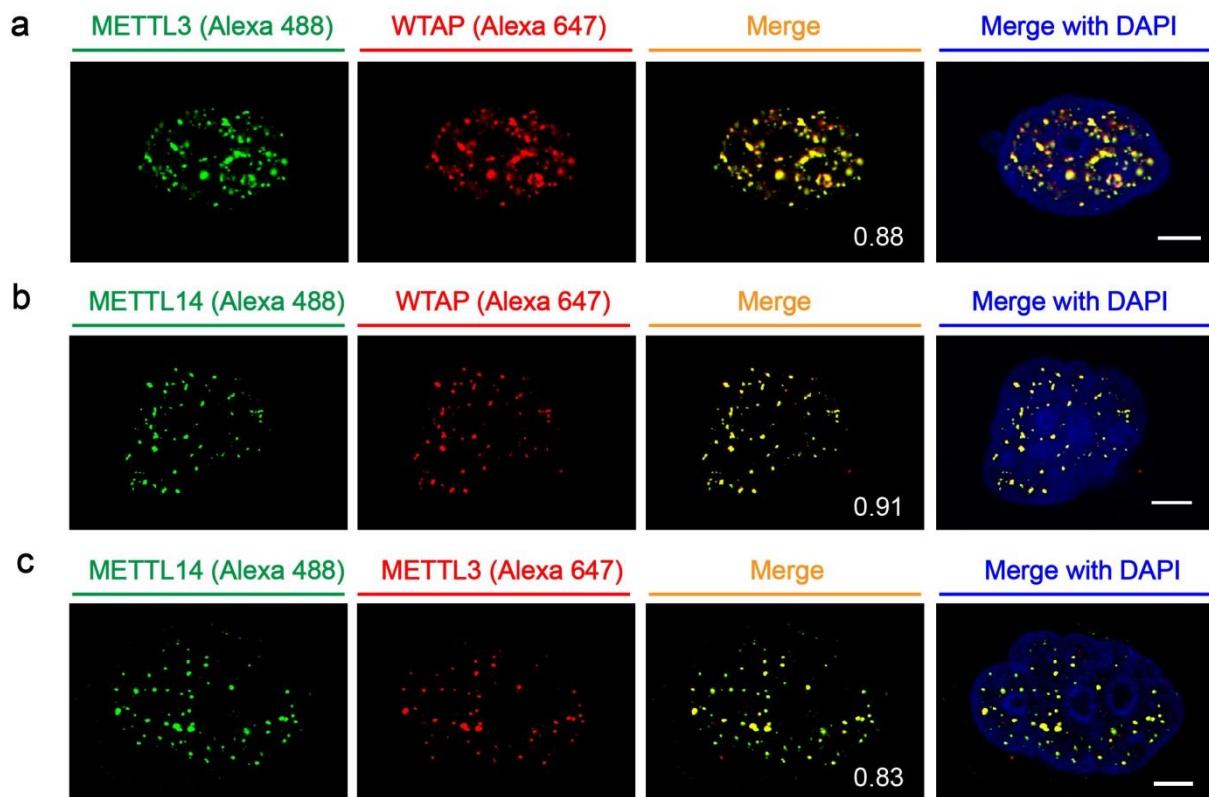
**Supplementary Figure 4 | Co-IP assays confirming interactions among METTL3, METTL14, and WTAP in HeLa cells.** (a) Flag-tagged METTL3, METTL14, and WTAP were expressed in HeLa cells, respectively, immunoprecipitated from cellular extracts via magnetic anti-Flag beads, and eluted by using a buffer containing 3× Flag peptide. The same HeLa cell line transfected with an empty pcDNA3 vector was used as control. All of the IP products were subjected to silver staining. The bands circled with solid line in the IP products were confirmed through LC-MS/MS protein identification (**Supplementary Tables 4–9**). (b) Western blotting was used to quantify the protein levels of METTL3, METTL14, and WTAP in each Input (whole cell lysate), co-IP (IP), and flow-through (FT) fraction. METTL3 showed a noteworthy enrichment in METTL14-IP fraction and vice versa, indicating that METTL3 and METTL14 exist as a complex inside cells. The interactions between WTAP and the other two proteins are weaker. (c) Western blotting showing that METTL4 was not in the METTL3-, METTL14-, and WTAP-IP fractions. (d) Methylation activity of each IP product, as well as the combination of METTL3-IP and METTL14-IP against probe 1. Error bars indicate mean ± s.d. ( $n = 6$ , three biological replicates × two technical replicates).



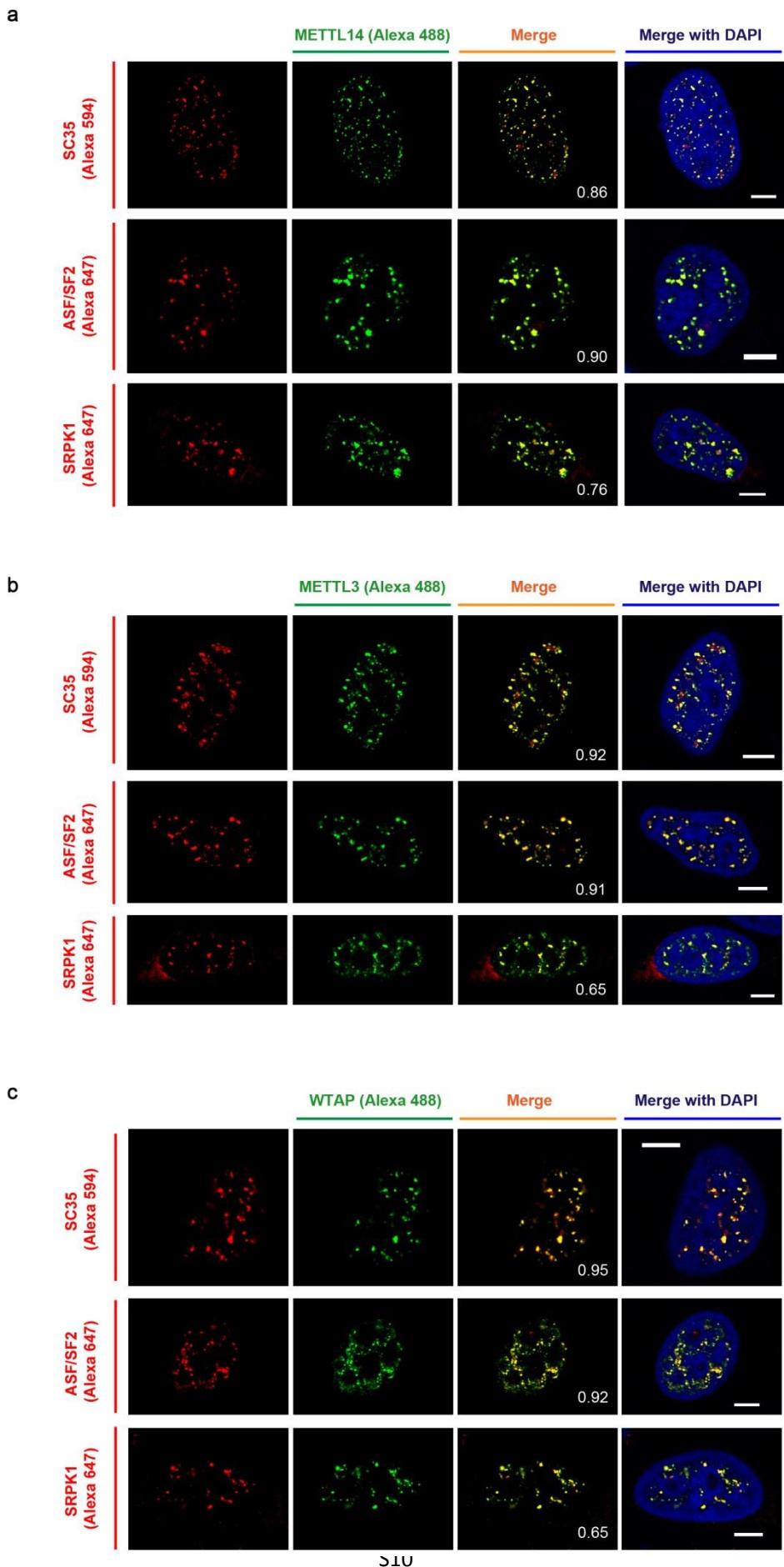
**Supplementary Figure 5 | Schematic illustration of  $N^6$ -adenosine methylation of ssRNA probes in the presence of tested proteins and isotope-labeled cofactor *S*-(5'-Adenosyl)-L-methionine- $d_3$  ( $d_3\text{-SAM}$ , 1). SAH is *S*-(5'-Adenosyl)-L-homocysteine.** We utilized  $d_3\text{-SAM}$  instead of common SAM as the cofactor to avoid potential contamination by RNA already bound to the recombinant protein during the purification process and for accurate mass spectrometry quantification.



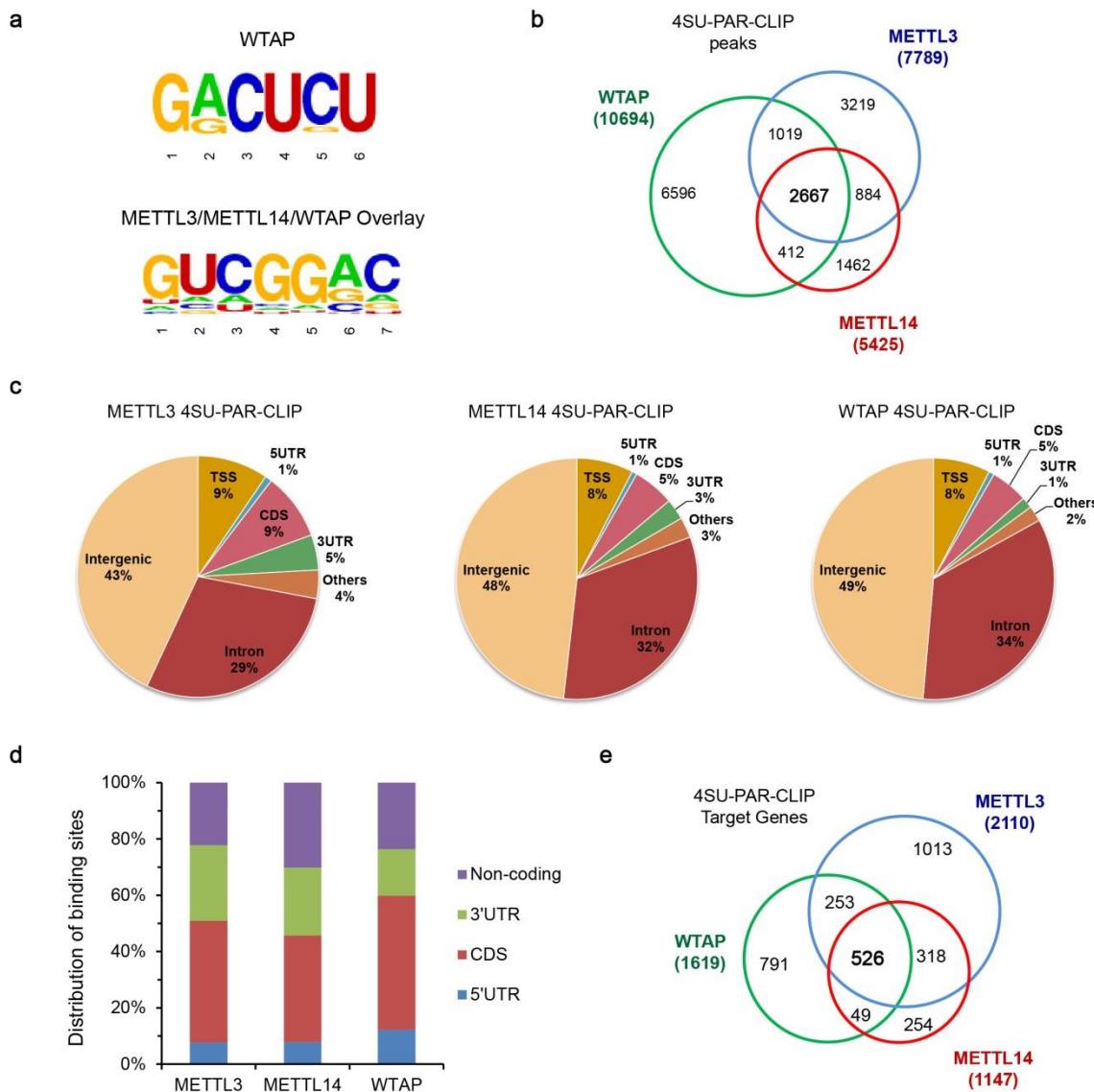
**Supplementary Figure 6 | RNA  $N_6$ -adenosine methylation by the METTL3-14 complex isolated from HeLa cell nuclear extract.** (a) Schematic illustration of the separation procedure of the HeLa cell nuclear extract. (b) The NE-A2-(19–21) fractions in Fig. 3a were combined and run on native PAGE. Western blotting showed co-migration of METTL3 and METTL14 as a complex.



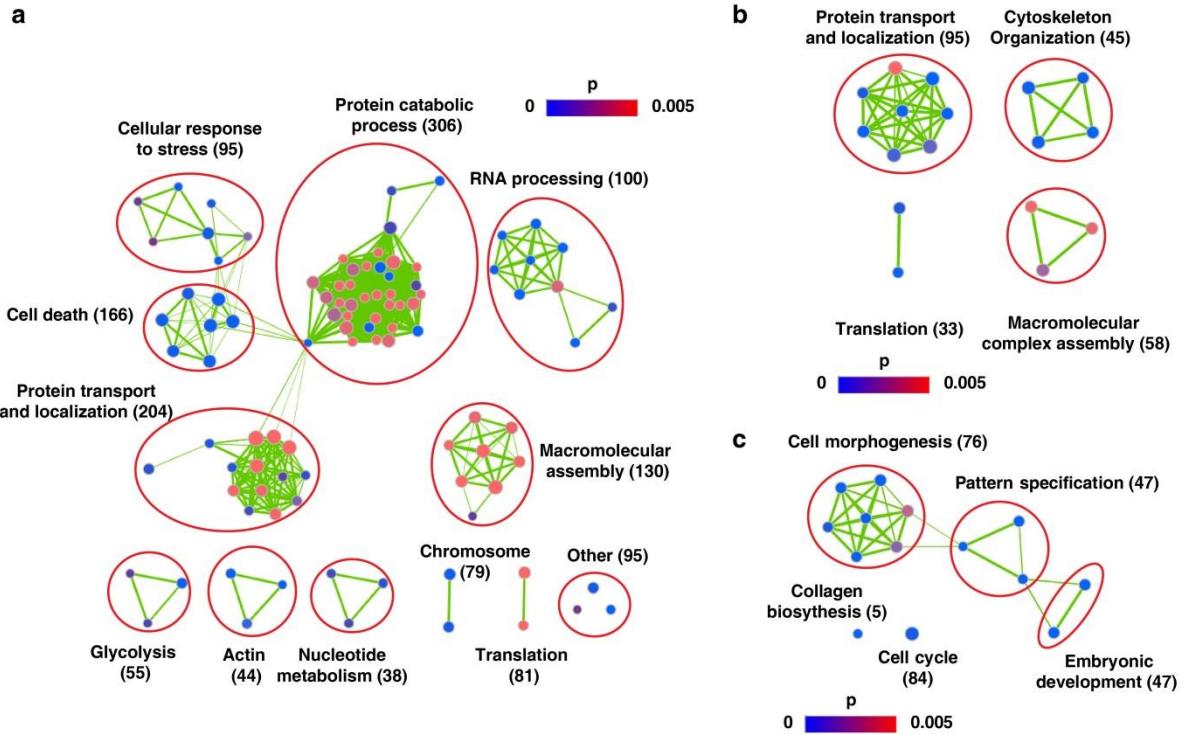
**Supplementary Figure 7 | Indirect immunofluorescence analysis showed that METTL3, METTL14, and WTAP are well co-localized with each other within cell nucleus.** (a) Fluorescence immunostaining of Flag-tagged METTL3 (green, anti-Flag, Alexa 488) and endogenous WTAP (red, anti-WTAP, Alexa 647). The yellow dots in the merged images indicate the co-localization of METTL3 and WTAP within cell nucleus. (b) Fluorescence immunostaining of Flag-tagged METTL14 (green, anti-Flag, Alexa 488) and endogenous WTAP (red, anti-WTAP, Alexa 647). (c) Fluorescence immunostaining of Flag-tagged METTL14 (green, anti-Flag, Alexa 488) and endogenous METTL3 (red, anti-METTL3, Alexa 647). The extent of co-localization was assessed by Pearson correlation coefficients of 488/647 channel pair as shown in the merged images. DAPI (blue): DNA staining. Scale bar: 5  $\mu$ m. Representative images from one of three independent experiments were shown for (a–c).



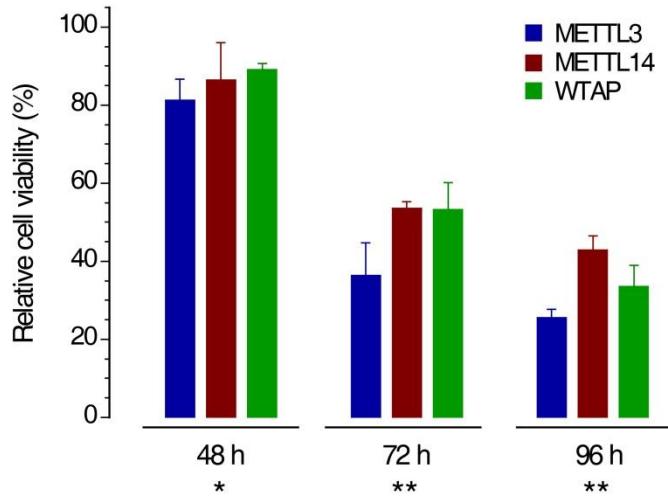
**Supplementary Figure 8 | Indirect immunofluorescence analysis showed that METTL3, METTL14, and WTAP co-localize with various pre-mRNA processing factors resided in the nuclear speckles.** (a–c) Fluorescence co-immunostaining of METTL3 (a, green, anti-Flag, Alexa 488), METTL14 (b, green, anti-Flag, Alexa 488), or WTAP (c, green, anti-Flag, Alexa 488) with various pre-mRNA processing factors including SC35-pi (red, anti-SC35-pi, Alexa 594), ASF/SF2 (red, anti-ASF/SF2, Alexa 647), and SRPK1 (red, anti-SRPK1, Alexa 647). The yellow dots in the merge images indicate the co-localization. The extent of co-localization was assessed by Pearson correlation coefficients of 488/594 and 488/647 channel pairs, respectively, which are shown in the merge images. DAPI (blue): DNA staining. Scale bar: 5  $\mu$ m. Representative images from one of the three independent experiments were shown for (a–c).



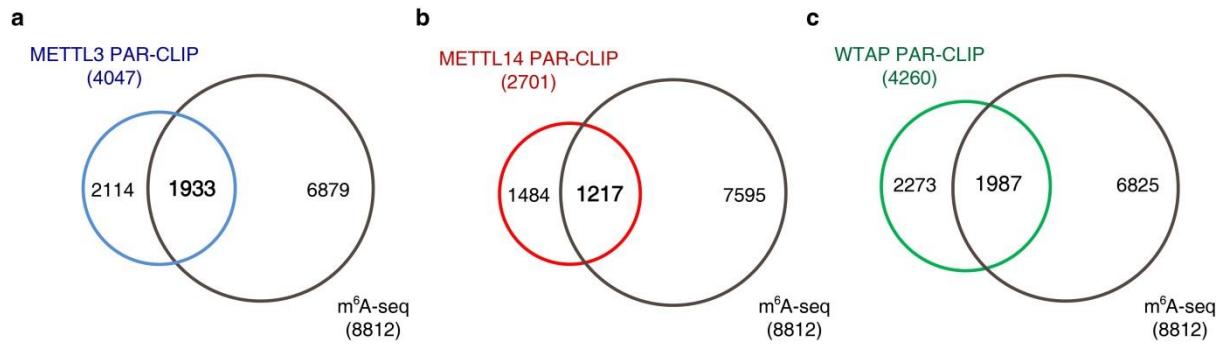
**Supplementary Figure 9 | Transcriptome-wide identification and analyses of the METTL3, METTL14, and WTAP binding sites on RNA based on PAR-CLIP.** (a) Consensus motifs identified within binding sites of WTAP ( $P = 1e-74$ ) and METTL3/METTL14/WTAP overlay ( $P = 1e-78$ ). (b) Venn diagram presenting the overlap of binding sites of the three proteins. METTL14 and METTL3 share an average of ~56% common binding sites while all three proteins have ~36% common binding sites. (c) Transcriptome-wide distribution of METTL3 (left panel), METTL14 (middle panel), and WTAP binding sites (right panel) in HeLa cells. A large fraction of the binding sites for all three proteins fall into intergenic regions (~43–49%) and introns (~29–34%). (d) Distribution of METTL3, METTL14, and WTAP binding sites along transcripts (sites assigned to 5'UTR, CDS, 3'UTR, and non-coding RNAs were included in this analysis). (e) Venn diagram presenting the overlap of coding transcripts bound by METTL3, METTL14, and WTAP revealed from the PAR-CLIP binding sites (sites assigned to introns were excluded).



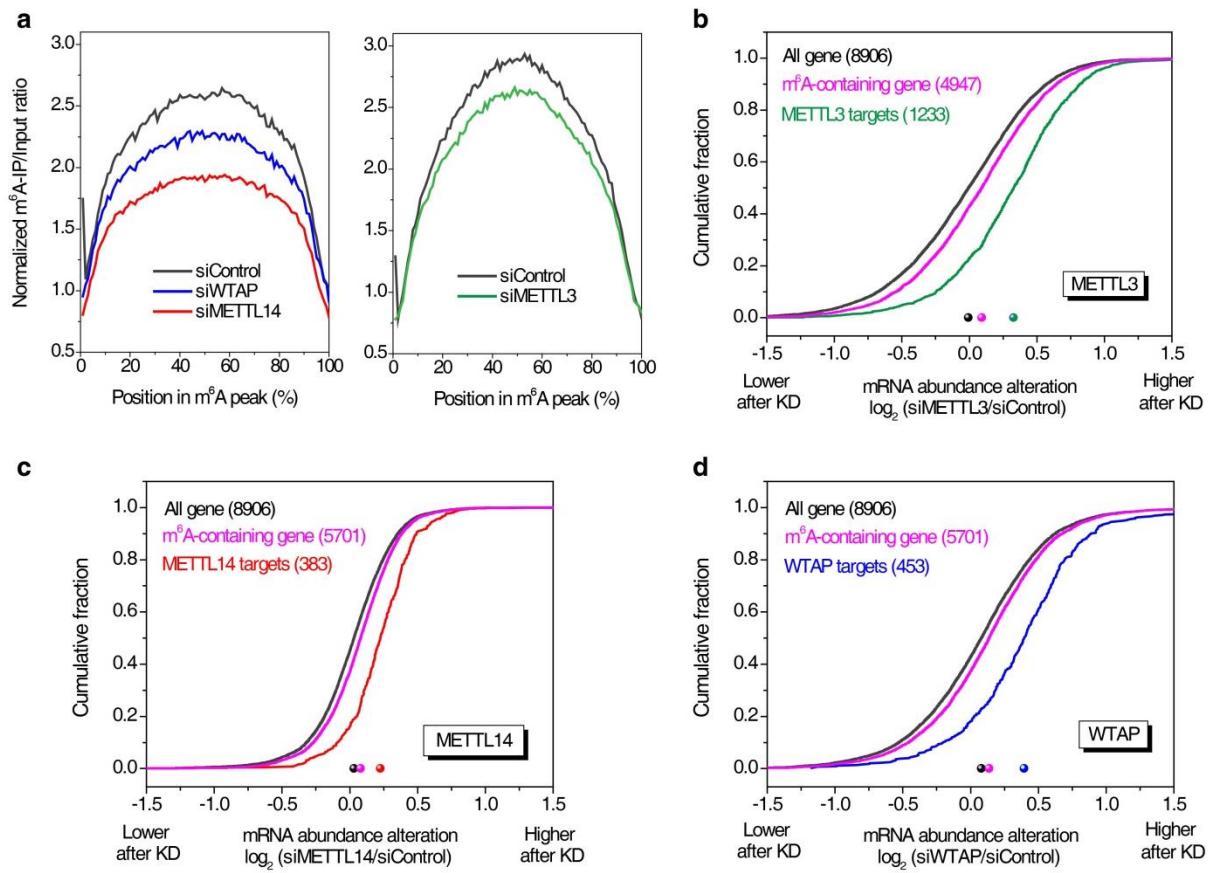
**Supplementary Figure 10 | Gene ontology (GO) and enrichment analysis of METTL3 (a), METTL14 (b), and WTAP (c) target transcripts identified via PAR-CLIP.** Each corresponding set of targets was subjected to DAVID GO analysis, and an enrichment map was constructed by using Cytoscape installed with the Enrichment Map Plugin. Each node denotes one enriched GO pathway ( $P < 0.005$ , FDR  $q < 0.1$ , overlap cutoff  $> 0.5$ ), with its color reflecting the  $P$  value. Node size is proportional to the total number of genes in each pathway. GO pathways of similar functions were sorted into one cluster, marked with circles and labels.



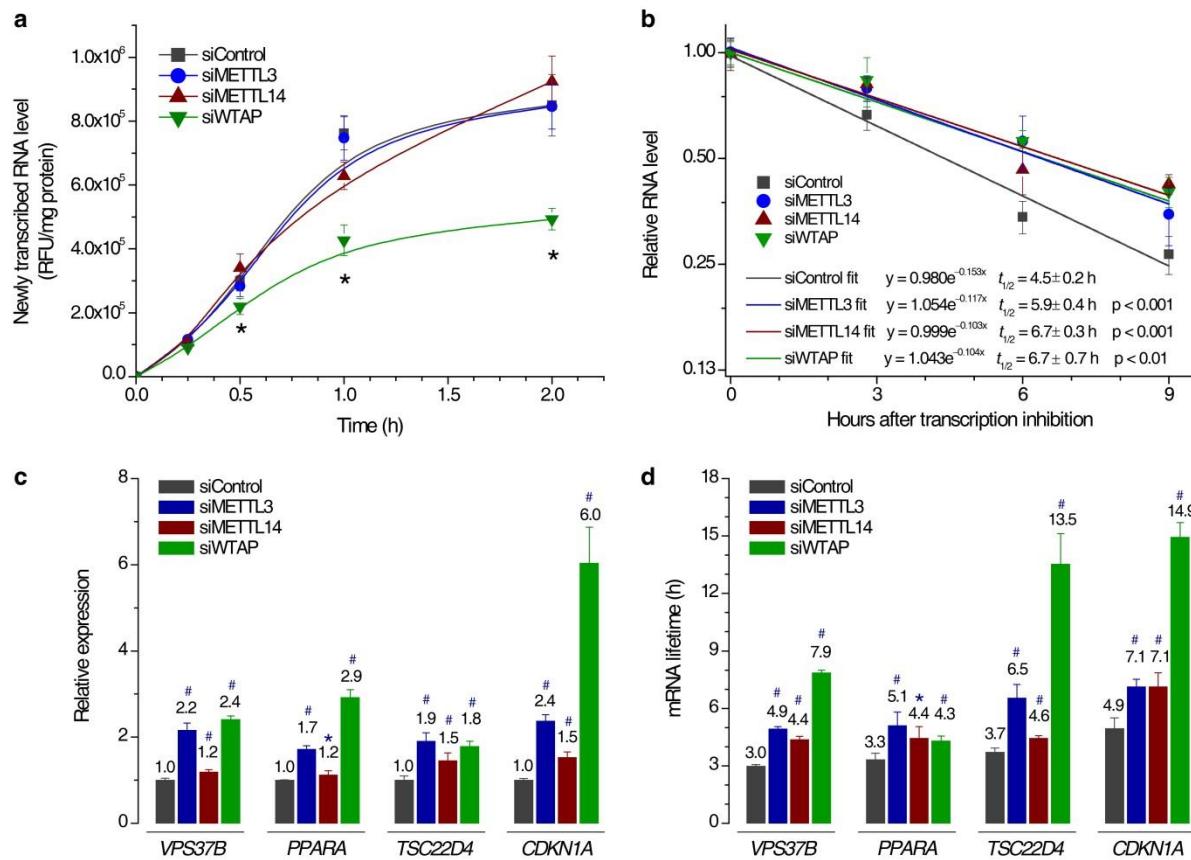
**Supplementary Figure 11 | Knockdown of METTL3, METTL14, and WTAP all led to reduced cell viability.** MTT assay provided experimental evidence for decreased cell viability upon knockdown of METTL3, METTL14, or WTAP. \*  $P < 0.05$  (versus control siRNA at 48 h post-transfection); and \*\*  $P < 0.0001$  (versus control siRNA at 72 h or 96 h post-transfection). Error bars indicate  $\pm$  SEM ( $n = 8$ , two biological replicates  $\times$  four technical replicates).



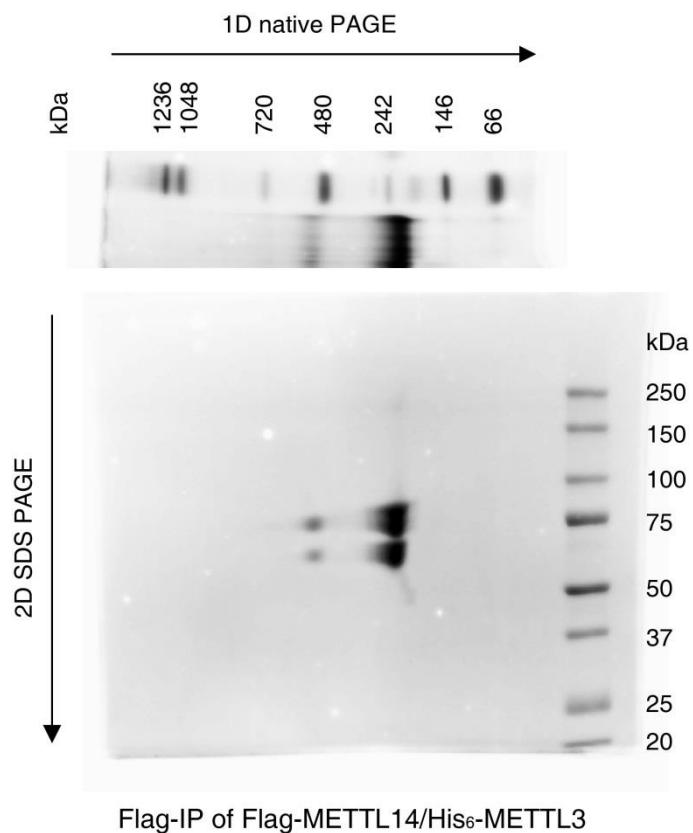
**Supplementary Figure 12 | Approximately 50% of METTL3, METTL14, or WTAP target genes identified by PAR-CLIP overlapped with the m<sup>6</sup>A-containing genes revealed from m<sup>6</sup>A-seq in the HeLa cells.**



**Supplementary Figure 13 | Identification and analyses of putative METTL3, METTL14, and WTAP target transcripts revealed by  $m^6\text{A}$ -seq.** (a) Distribution of normalized  $m^6\text{A}$ -IP/ $m^6\text{A}$ -Input ratio across the window of the  $m^6\text{A}$  peaks upon knockdown of METTL3, METTL14, and WTAP, respectively. (b-d) Comparisons of changes of the average abundance of all transcripts with those of the corresponding  $m^6\text{A}$ -containing transcripts as well as transcripts containing  $m^6\text{A}$  affected by the knockdown of METTL3 (b), METTL14 (c), or WTAP (d). Overall, the reduced  $m^6\text{A}$  methylation on transcripts affected by METTL3, METTL14, or WTAP led to increased abundance of the corresponding mRNAs as compared to all transcripts inside the HeLa cells.

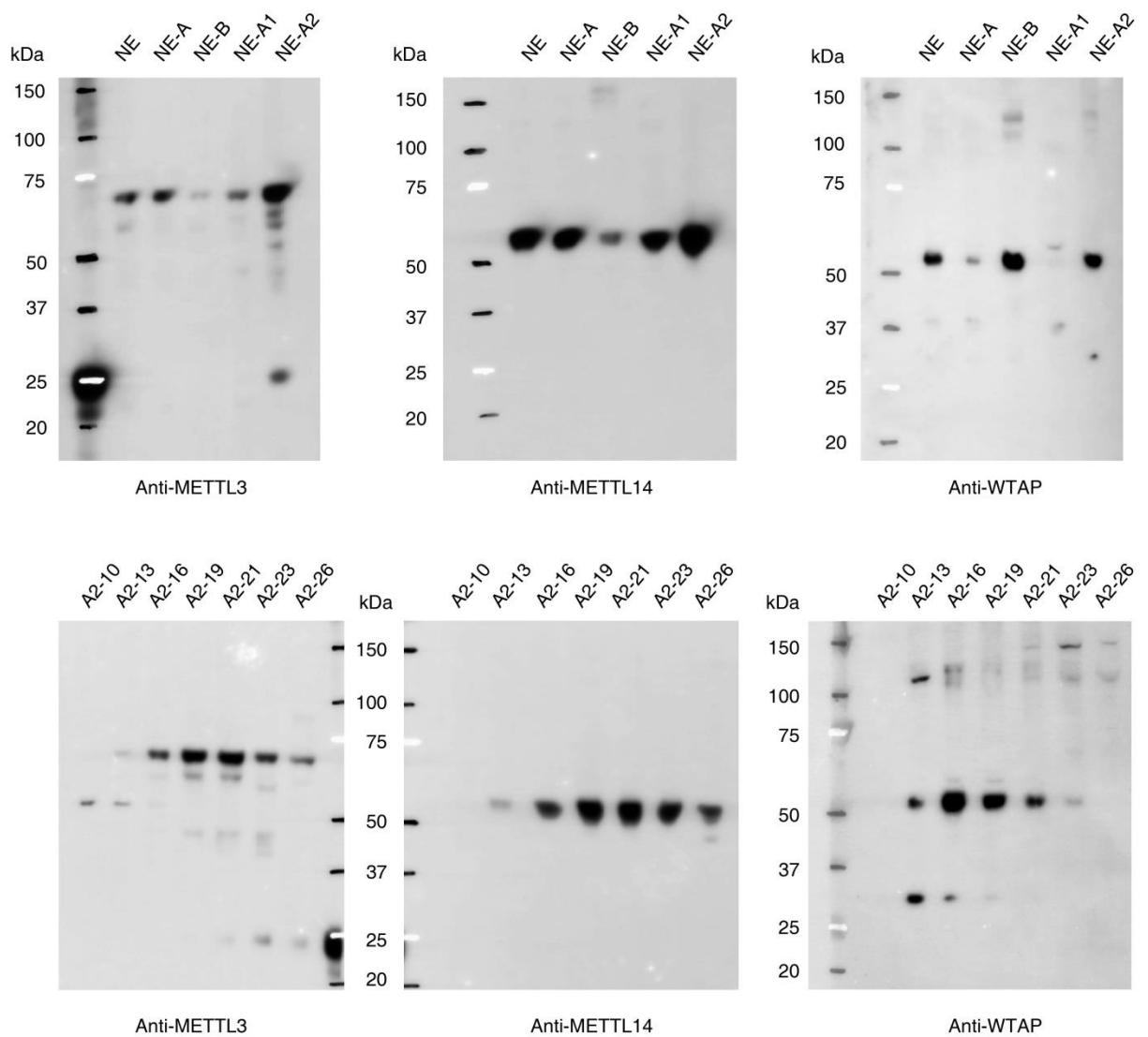


**Supplementary Figure 14 | Nascent mRNAs showed increased lifetimes with the overall reduced methylation.** (a) Time dependence of the levels of newly transcribed RNAs in samples of siMETTL3, siMETTL14, and siWTAP versus siControl. While knockdowns of METTL3 and METTL14 hardly affected the global RNA transcription rate, WTAP knockdown decreased the total nascent RNA synthesis by more than 30% (\*  $P < 0.001$ , Student's  $t$ -test). WTAP seems to affect nascent RNA synthesis, perhaps through its splicing function. (b) Average lifetimes of nascent RNA in samples of siMETTL3, siMETTL14, and siWTAP versus siControl. The overall increased mRNA lifetimes compared to siControl were observed in each case with individual knockdown of the three proteins. The  $P$  value is calculated between control and specific knockdown sample by Student's  $t$ -test. Error bars shown in (a and b) indicate mean  $\pm$  s.d.,  $n = 10$  (two biological replicates  $\times$  five technical replicates). (c and d) Relative changes (c) and lifetimes (d) of selected *VPS37B*, *PPARA*, *TSC22D4*, and *CDKN1A* mRNAs in samples of siMETTL3, siMETTL14, and siWTAP versus siControl. *GAPDH* was chosen as an internal control. *VPS37B* is a common m<sup>6</sup>A target of METTL3, METTL14, and WTAP; *PPARA* is a common m<sup>6</sup>A target of METTL3 and METTL14; *TSC22D4* is an m<sup>6</sup>A target of METTL3; *CDKN1A* is an m<sup>6</sup>A target of WTAP. \*  $P < 0.05$ , and #  $P < 0.01$  (Student's  $t$ -test, calculated between control and specific knockdown sample). Error bars indicate mean  $\pm$  s.d.,  $n = 4$  (two biological replicates  $\times$  two technical replicates).



Flag-IP of Flag-METTL14/His<sub>6</sub>-METTL3

**Supplementary Figure 15 | Full gel images of data shown in Figure 1c.** Coomassie staining of two-dimensional native/SDS PAGE of the Flag-IP product from insect cells co-expressing Flag-METTL14/His<sub>6</sub>-METTL3.



**Supplementary Figure 16 | Full gel images of western blotting shown in Figure 3a.**

**Supplementary Table 1. siRNA sequences used in this work**

siRNA	Target sequences (5' to 3')
siMETTL3	CTGCAAGTATGTTCACTATGA
siMETTL3-a	AGGAGCCAGCCAAGAAATCAA
siMETTL14	AAGGATGAGTTAATAGCTAAA
siMETTL14-a	TGGTGCCGTGTTAAATAGCAA
siMETTL4	ATGGCAGAACAAATCAGTTAA
siMETTL4-a	AAGCCCTACGAAGGTCTTATA
siWTAP	AAGCTTGGAGGGCAAGTACA
siWTAP-a	AAGGTTCGATTGAGTGAAACA
siWTAP-b	GGGCAAGTACACAGATCTTAA

**Supplementary Table 2. Primers used for the RT-qPCR that span exon-exon junction**

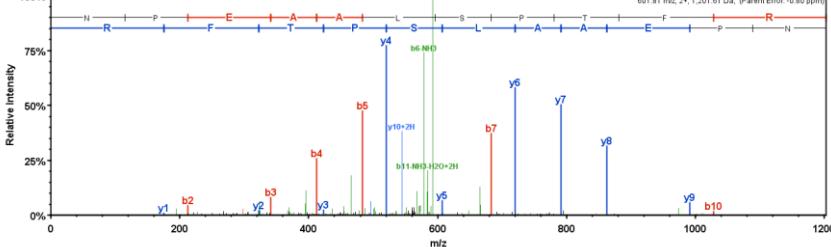
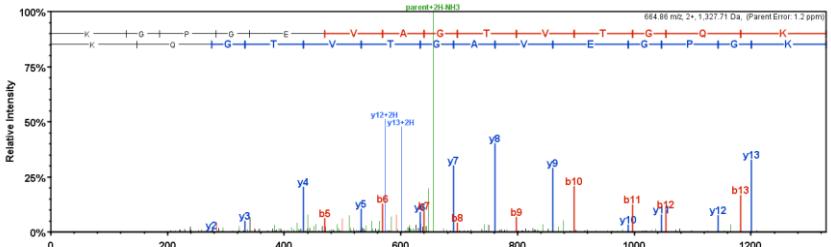
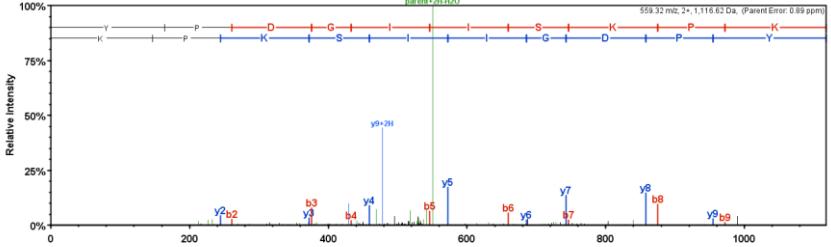
<i>Target gene</i>	Left primer (5' to 3')	Right primer (5' to 3')
<i>METTL3</i>	CAAGCTGCACCCAGACGAA	GCTTGGCGTGTGGTCTTT
<i>METTL14</i>	AGAAAATTGCAGGGCTTCCT	TCTTCTTCATATGGCAAATTTCTT
<i>METTL4</i>	TGCAACCACCTCTAAACTATAGGA AA	TTTGGAGCAGCCAATTAGG
<i>WTAP</i>	TTCCCCAAGAACGGTCGATTG	TGCAGACTCCTGCTGTTGTT
<i>PPARA</i>	GGATGCTGGTAGCGTATGGA	GGACGATCTCCACAGCAAAT
<i>TSC22D4</i>	CAGAAGAGATGGGGCAGGT	TCATCGTCGCTGTCTAGGTG
<i>CDKN1A</i>	CACCTCACCTGCTCTGCTG	AATCTGTCATGCTGGTCTGC
<i>VPS37B</i>	TGCAGAACGATGGAGGGAGACA	GGCTTCAAAGAGAACCTGGA

**Supplementary Table 3. Primers used in the cloning of METTL3, METTL14, and WTAP**

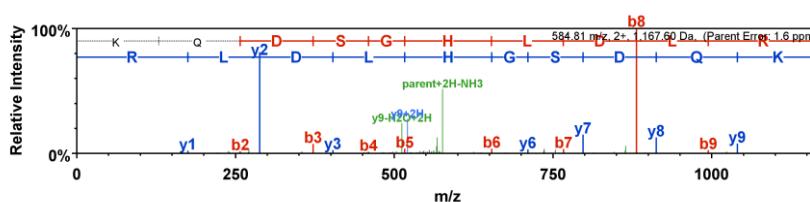
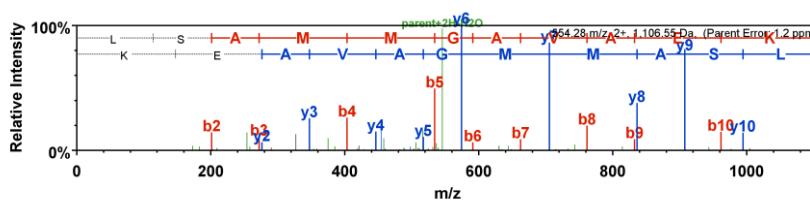
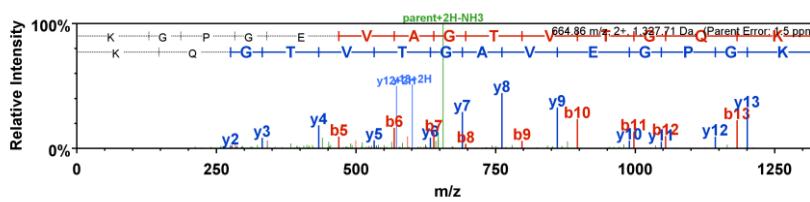
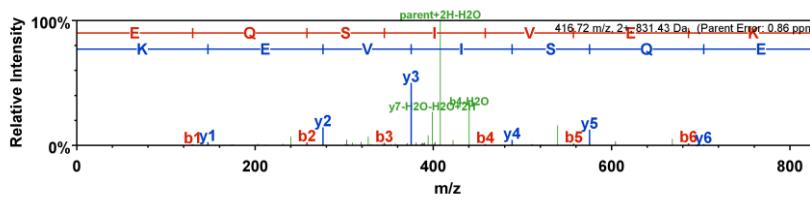
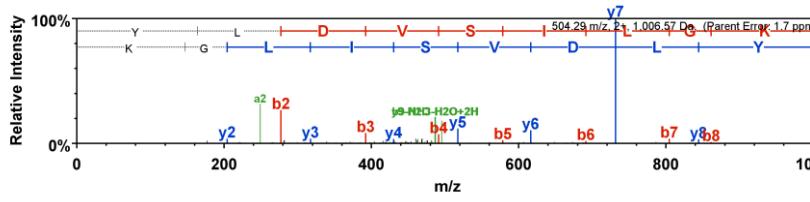
<b>Primer</b>	Sequence (5' to 3'): Red: Restriction enzyme site; Green: Flag-tag; Purple: His-tag; Orange: TEV cleavage site.
	<b>For expression in High Five insect cell</b>
GST-METTL3-F	AATT <b>GTCGAC</b> AA <b>GAGAACCTGTACTTCCAATCC</b> ATGTCGGACACGTGGAGCT
GST-METTL3-R	AATT <b>AAGCTT</b> CTATAAATTCTTAGGTTAGAGATGATAC
	<b>For expression in SF9 insect cell</b>
His-METTL3-F	AATT <b>GTCGAC</b> ATG <b>CATCATCACCAC</b> TCGGACACGTGGAGCT
His-METTL3-R	AATT <b>AAGCTT</b> CTA TAAATTCTTAGGTTAGAGATGATAC
Flag-METTL3-F	AATT <b>GTCGAC</b> ATG <b>GATTACAAGGATGACGATGACAAG</b> TCGGACACGTGGAGCT
Flag-METTL3-R	AATT <b>AAGCTT</b> CTA TAAATTCTTAGGTTAGAGATGATAC
His-METTL14-F	AATT <b>GGATCC</b> ATG <b>CATCATCACCAC</b> GATAGCCGCTTGCAGGA
His-METTL14-R	ATAAGAAT <b>GCGGCCGC</b> TTA TCGAGGTGGAAAGCCAC
Flag-METTL14-F	AATT <b>GGATCC</b> ATG <b>GATTACAAGGATGACGATGACAAG</b> GATAGCCGCTTGCAGGA
Flag-METTL14-R	ATAAGAAT <b>GCGGCCGC</b> TTA TCGAGGTGGAAAGCCAC
His-WTAP-F	AATT <b>GGATCC</b> ATG <b>CATCATCACCAC</b> ACCAACGAAGAACCTCTTCC
His-WTAP-R	ATAAGAAT <b>GCGGCCGC</b> TTA CAAA ACTGAACCCTGTACATTAC
Flag-WTAP-F	AATT <b>GGATCC</b> ATG <b>GATTACAAGGATGACGATGACAAG</b> ACCAACGAAGAACCTCTTC C
Flag-WTAP-R	ATAAGAAT <b>GCGGCCGC</b> TTA CAAA ACTGAACCCTGTACATTAC
	<b>For over-expression in HeLa cells</b>
Flag-METTL3-F	AATT <b>AAGCTT</b> ATGG <b>GATTACAAGGATGACGATGACAAG</b> TCGGACACGTGGAGCT
Flag-METTL3-R	AATT <b>TCTAGA</b> CTATAAATTCTTAGGTTAGAGATGATAC
Flag-METTL14-F	AATT <b>GGATCC</b> ATGG <b>GATTACAAGGATGACGATGACAAG</b> GATAGCC GCTTGCAGGA
Flag-METTL14-R	AATT <b>CTCGAG</b> TTATCGAGGTGGAAAGCCAC
Flag-WTAP-F	AATT <b>GGATCC</b> ATGG <b>GATTACAAGGATGACGATGACAAG</b> ACCAACG AAGAACCTCTTCC
Flag-WTAP-R	AATT <b>CTCGAG</b> TTACAAA <b>ACTGAACCCTGTACATTACA</b>

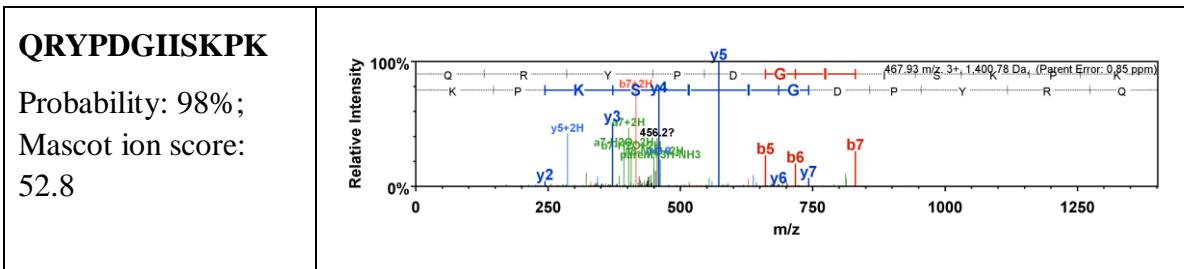
**Supplementary Table 4. LC-MS/MS protein identification of METTL3 from the WTAP**

IP product circled with the solid line in Supplementary Fig. 4a

METTL3 (100%), 64,475.0 Da (580 aa) 3 exclusive unique peptides, 4 exclusive unique spectra, 4 total spectra, 35/580 amino acids (6% coverage)	
Unique peptides	Unique MS spectra
<b>NPEAALSPTR</b> Probability: 100%; Mascot ion score: 62.9	 <p>Mass spectrum showing Relative Intensity (0% to 100%) vs m/z (0 to 1200). The base peak is at m/z 601.81, labeled as parent + 2H2O. Other labeled peaks include b2, b3, b4, b5, y1, y2, y3, y4, y5, y6, y7, y8, y9, and b10.</p>
<b>KGPGEVAGTVTG</b> <b>QK</b> Probability: 100%; Mascot ion score: 78.5	 <p>Mass spectrum showing Relative Intensity (0% to 100%) vs m/z (0 to 1200). The base peak is at m/z 684.86, labeled as parent + 2H NH3. Other labeled peaks include y2, y3, y4, y5, y6, y7, y8, y9, b5, b6, b7, b8, b9, b10, b11, b12, y10, y11, y12, and y13.</p>
<b>YPDGIISKPK</b> Probability: 100%; Mascot ion score: 69.3	 <p>Mass spectrum showing Relative Intensity (0% to 100%) vs m/z (0 to 1000). The base peak is at m/z 558.32, labeled as parent + 2H2O. Other labeled peaks include y2, y3, y4, y5, y6, y7, y8, b5, b6, b7, b8, y9, and b10.</p>

**Supplementary Table 5. LC-MS/MS protein identification of METTL3 from the METTL14 IP product circled with the solid line in Supplementary Fig. 4a**

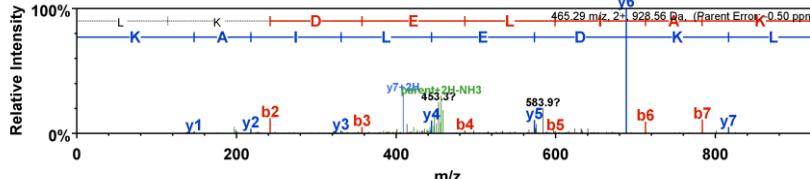
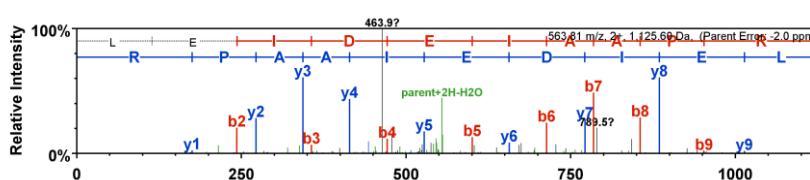
<b>METTL3 (100%), 64,475.0 Da (580 aa)</b> <b>9 exclusive unique peptides, 13 exclusive unique spectra, 13 total spectra, 63/580 amino acids (11% coverage)</b>	
 <pre> M S D T W S I Q A H K K Q L D S L R E R L Q R R R K Q D S G H L D L R N P E A A L S P T F R S D S P V P T A P T S G G P K P S T A S A V P E L A T D P E L E K K L L H H L S D L A L T L P T D A V S I C L A I S T P D A P A T Q D G V E S L L Q K F A A Q E L I E V K R G L L Q D D A H P T L V T Y A D H S K L S A M M G A V A E K K G P G E V A G T V G Q K R R A E Q D S T T V A A F A S S L V S G L N S S A S E P A K E P A K K S R K H A A S D V D L E I E S L L N Q Q S T K E Q Q S K K V S Q E I L E L L N T T T A K E Q S I V E K F R S P G R A Q V Q E F C D Y G T K E E C M K A S D A D R P C R K L H F R R I I N K H T D E S L G D C S F L N T C F H M D T C K Y V H Y E I D A C M D S E A P G S K D H T P S Q E L A L T Q S V G G D S S A D R L F P P Q W I C C D I R Y L D V S I L G K F A V V M A D P P W D I H M E L P Y G T L T D D E M R R L N I P V L Q D D G F L F L W V T G R A M E L G R E C L N L W G Y E R V D E I I W V K T N Q L Q R I I R T G R T G H W L N H G K E H C L V G V K G N P Q G F N Q Q G L D C D V I V A E V R S T S H K P D E I Y G M I E R L S P G T R K I E L F G R P H N V Q P N W I T L G N Q L D G I H L L D P D V V A R K Q R Y P D G I I S K P K N L </pre>	
Unique peptides	Unique MS spectra
<b>KQDSGHLDLR</b> Probability: 99%; Mascot ion score: 79.5	 <p>Relative Intensity</p> <p>m/z</p> <p>584.81 m/z 2+, 1.167.60 Da. (Parent Error: 1.6 ppm)</p> <p>parent+2H-NH3</p>
<b>LSAMMGAVAEK</b> Probability: 99%; Mascot ion score: 95.1	 <p>Relative Intensity</p> <p>m/z</p> <p>584.28 m/z 2+, 1.106.55 Da. (Parent Error: 1.2 ppm)</p> <p>parent+2H-NH3</p>
<b>KGPGEVAGTVTGQK</b> Probability: 99%; Mascot ion score: 106.0	 <p>Relative Intensity</p> <p>m/z</p> <p>664.86 m/z 2+, 1.137.71 Da. (Parent Error: 1.5 ppm)</p> <p>parent+2H-NH3</p>
<b>EQSIVEK</b> Probability: 90%; Mascot ion score: 45.9	 <p>Relative Intensity</p> <p>m/z</p> <p>416.72 m/z 2+, 831.43 Da. (Parent Error: 0.86 ppm)</p> <p>parent+2H-H2O</p>
<b>YLDVSLGK</b> Probability: 99%; Mascot ion score: 49.2	 <p>Relative Intensity</p> <p>m/z</p> <p>504.29 m/z 2+, 1.006.57 Da. (Parent Error: 1.7 ppm)</p> <p>y9-NH2-H2O+2H</p>



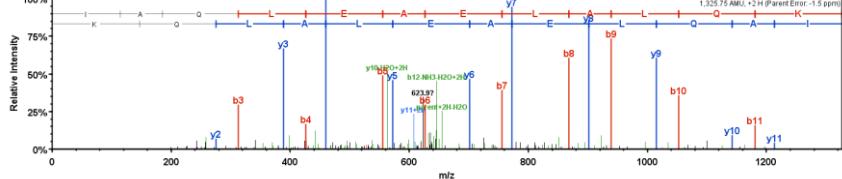
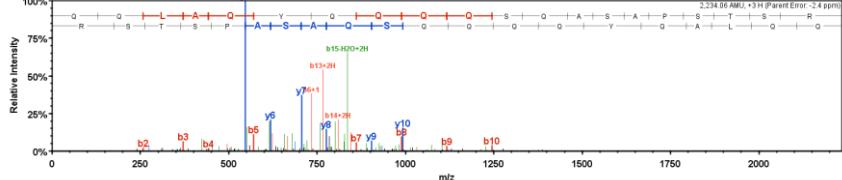
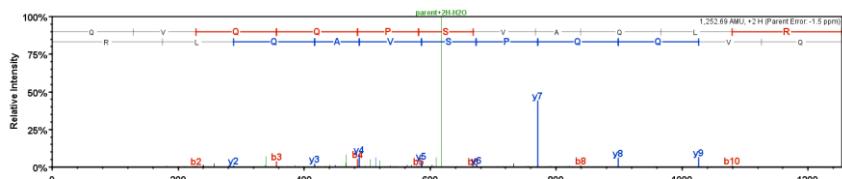
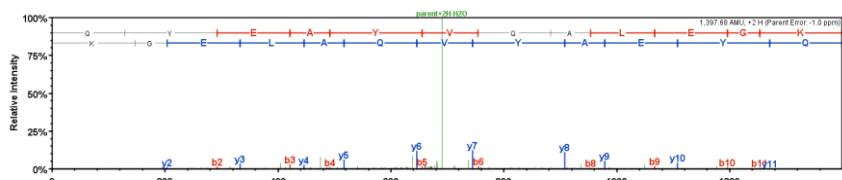
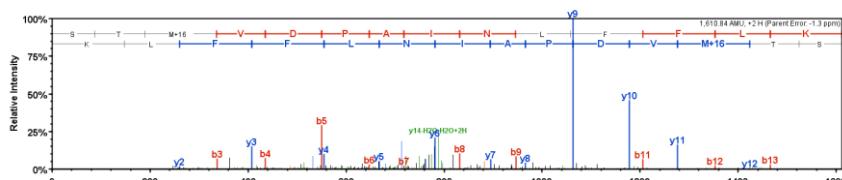
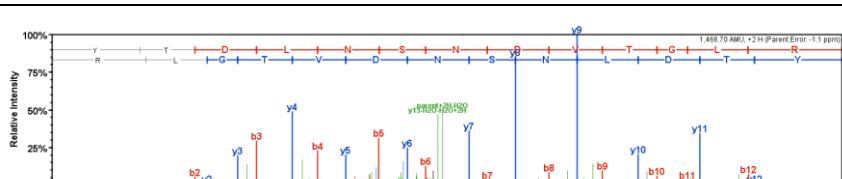
**Supplementary Table 6. LC-MS/MS protein identification of METTL14 from the WTAP IP product circled with the solid line in Supplementary Fig. 4a**

<p>METTL14 (84%), 52,152.7 Da (456 aa) 1 exclusive unique peptides, 1 exclusive unique spectra, 1 total spectra, 8/456 amino acids (2% coverage)</p> <pre> M D S R L Q E I R E   R Q K L R R Q L L A   Q Q L G A E S A D S   I G A V L N S K D E   Q R E I A E T R E T   C R A S Y D T S A P   N A K R K Y L D E G E T D E D K M E E Y   K D E L E M Q Q D E   E N L P Y E E E I Y   K D S S T F L K G T   Q S L N P H N D Y C   Q H F V D T G H R P   Q N F I R D V G L A D R F E E Y P K L R   E L I R L <b>L K D E L I A K</b>   A K   S N T P P M Y L   Q A D I E A P R S F   E L T P K F D V I L   L E P P L E E Y Y R   E T G I T A N E K C W T W D D I M K L E   I D E I A A P R S F   I F L W C G S G E G   L D L G R V C L R K   W G Y R R C E D I C   W I K T N K N N P G   K T K T L D P K A V F Q R T K E H C L M   G I K G T V K R S T   D G D F I H A N V D   I D L I I T E E P E   I G N I E K P V E I   F H I I E H F C L G   R R R L H L F G R D S T I R P G W L T V   G P T L T N S N Y N   A E T Y A S Y F S A   P N S Y L T G C T E   E I E R L R P K S P   P P K S K S D R G G   G A P R G G G R G G T S A G R G R E R N   R S N F R G E R G G   F R G G R G G A H R   G G F P P R </pre>	
<b>Unique peptides</b>	<b>Unique MS spectra</b>
<b>LKDELIAK</b> Probability: 99%; Mascot ion score: 44.3	

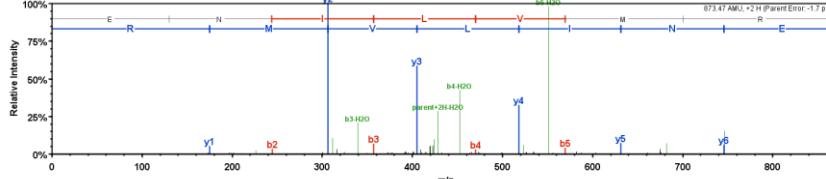
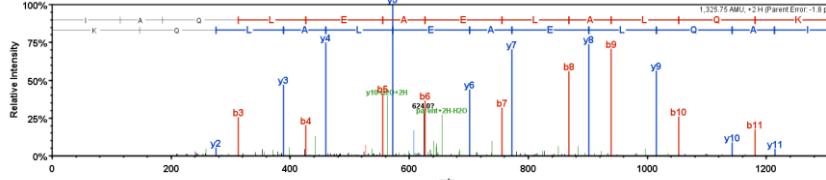
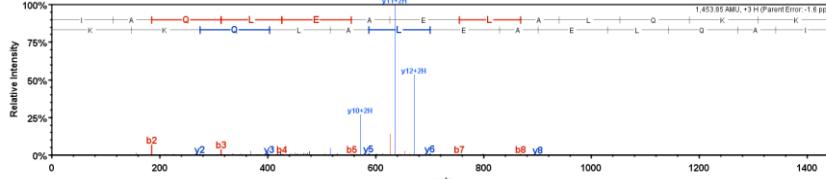
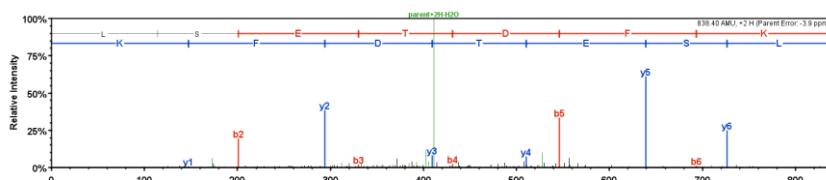
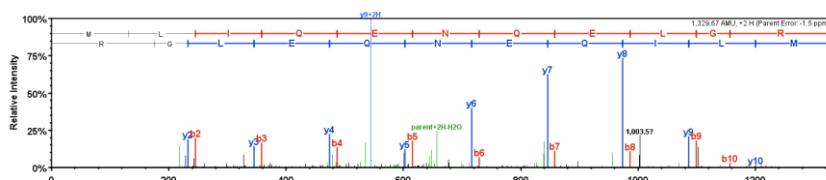
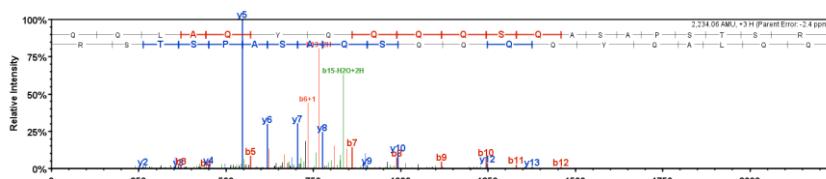
**Supplementary Table 7. LC-MS/MS protein identification of METTL14 from the METTL3 IP product circled with the solid line in Supplementary Fig. 4a**

<b>METTL14 (100%), 52,152.7 Da (456 aa) 2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 18/456 amino acids (4% coverage)</b>	
<b>Unique peptides</b>	<b>Unique MS spectra</b>
<b>LKDELIAK</b> Probability: 99%; Mascot ion score: 38.8	 <p>Mass spectrum showing Relative Intensity (0% to 100%) versus <math>m/z</math> (0 to 800). The base peak is at <math>m/z</math> 465.29. Other labeled peaks include <math>y_1</math>, <math>y_2</math>, <math>b_2</math>, <math>y_3</math>, <math>b_3</math>, <math>y_4</math>, <math>b_4</math>, <math>y_5</math>, <math>b_5</math>, <math>y_6</math>, <math>b_6</math>, <math>y_7</math>, <math>b_7</math>, and a water cluster at <math>m/z</math> 453.37 and 583.97.</p>
<b>LEIDEIAAPK</b> Probability: 100%; Mascot ion score: 69.7	 <p>Mass spectrum showing Relative Intensity (0% to 100%) versus <math>m/z</math> (0 to 1000). The base peak is at <math>m/z</math> 463.97. Other labeled peaks include <math>y_1</math>, <math>b_2</math>, <math>y_2</math>, <math>b_3</math>, <math>y_3</math>, <math>y_4</math>, <math>b_4</math>, <math>y_5</math>, <math>b_5</math>, <math>y_6</math>, <math>b_6</math>, <math>y_7</math>, <math>b_7</math>, <math>y_8</math>, <math>b_8</math>, <math>y_9</math>, and <math>b_9</math>.</p>

**Supplementary Table 8. LC-MS/MS protein identification of WTAP from the METTL3 IP product circled with the solid line in Supplementary Fig. 4a**

gl 10334526 (100%), 43,412.5 Da WTAP protein [Homo sapiens] 6 unique peptides, 6 unique spectra, 6 total spectra, 82/388 amino acids (21% coverage)	
Unique peptides	Unique MS spectra
<b>IAQLEALALQK</b> Probability: 95%; Mascot ion score: 98.9	
<b>QQLAQYQQQQS</b> <b>QASAPTSR</b> Probability: 95%; Mascot ion score: 48.2	
<b>QVQQPSVAQLR</b> Probability: 95%; Mascot ion score: 50.2	
<b>QYEAYVQALEGK</b> Probability: 95%; Mascot ion score: 74.4	
<b>STMVDPAINLFFL</b> <b>K</b> Probability: 95%; Mascot ion score: 70.5	
<b>YTDLNSNDVTGL</b> <b>R</b> Probability: 95%; Mascot ion score: 91.2	

**Supplementary Table 9. LC-MS/MS protein identification of WTAP from METTL14 IP product circled with solid line in Supplementary Fig. 4a**

gi 10334526 (100%), 43,412.5 Da WTAP protein [Homo sapiens] 11 unique peptides, 11 unique spectra, 11 total spectra, 109/388 amino acids (28% coverage)	
<pre> E E P L P K K V M A R   S E T D F K Y V M A R   D E L I L R W K Q Y   E A Y V Q A L E G K   Y T D L N S N D V T   G L R E S E E K L K   Q Q Q Q E S A R R E N I L V M R L A T K   E Q E M Q E C T T Q   I Q Y L K Q V Q Q P   Q V A Q L R S T M V   D P A I N L F F L K   M K G E L E Q T K D   K L E Q A Q N E L S A W K F T P D S Q T   G K K L M A K C R M   L I Q E N Q E L G R   Q L S Q G R I A Q L   E A E L A L Q K Y   S E E L K S S Q D E   L N D F I I Q L D E E V E G M Q S T I L   V L Q Q Q L K E T R   Q Q L A Q Y Q Q Q Q   S Q A S A P S T S R   T T A S E P V E Q S   E A T S K D C S R L   T N G P S N G S S S R Q R T S G S G F H   R E G N T T E D D F   P S S P G N G N K S   S N S S E E R T G R   G G S G Y V N Q L S   A G Y E S V D S P T   G S E N S L T H Q S N D T D S S H D P Q   E E K A V S G K G N   R T V G S R H V Q N   G L D S S V N V </pre>	
Unique peptides	Unique MS spectra
<b>ENILVMR</b> Probability: 95%; Mascot ion score: 45.8	
<b>IAQLEAELALQK</b> Probability: 95%; Mascot ion score: 123.5	
<b>IAQLEAELALQK</b> <b>K</b> Probability: 95%; Mascot ion score: 33.0	
<b>LSETDFK</b> Probability: 95%; Mascot ion score: 36.4	
<b>MLIQENQELGR</b> Probability: 95%; Mascot ion score: 73.3	
<b>QQLAQYQQQS</b> <b>QASAPTSR</b> Probability: 95%; Mascot ion score: 49.6	

<p><b>QVQQPSVAQLR</b></p> <p>Probability: 95%; Mascot ion score: 54.1</p>	<p>parent +2H H<sub>2</sub>O 1,252.69 AMU, +2 H (Parent Error: -1.6 ppm)</p>
<p><b>QYEAYVQALEGK</b></p> <p>Probability: 95%; Mascot ion score: 76.4</p>	<p>parent +2H H<sub>2</sub>O 1,397.68 AMU, +2 H (Parent Error: -1.2 ppm)</p>
<p><b>RENILVMR</b></p> <p>Probability: 95%; Mascot ion score: 41.1</p>	<p>parent +2H H<sub>2</sub>O 1,029.57 AMU, +2 H (Parent Error: -2.1 ppm)</p>
<p><b>STMVDPAINLFFL K</b></p> <p>Probability: 95%; Mascot ion score: 60.4</p>	<p>parent +2H H<sub>2</sub>O 1,610.84 AMU, +2 H (Parent Error: -1.8 ppm)</p>
<p><b>YTDLNSNDVTGL</b></p> <p>Probability: 95%; Mascot ion score: 96.0</p>	<p>parent +2H H<sub>2</sub>O 1,466.70 AMU, +2 H (Parent Error: -1.3 ppm)</p>

**Supplementary Table 10. PAR-CLIP sequencing data summary<sup>a</sup>**

Sample name	Total reads	Trimmed reads <sup>b</sup>	Mappable reads	Alignment ratio <sup>c</sup>	Number of PAR-CLIP reads group	Number of PAR-CLIP binding clusters
4SU-M3-1	42994498	38905832	32087911	82.48%	54968	7664
4SU-M3-2	23404657	15823788	12790921	80.83%	29363	4293
4SU-M14-1	10208273	7387670	5424089	73.42%	19885	3163
4SU-M14-2	23878323	22021415	15912529	72.26%	29604	5326
4SU-WTAP-1	47159271	30289349	24453403	80.73%	114623	10964
4SU-WTAP-2	23248339	8967225	6885893	76.79%	38257	3629

<sup>a</sup> Two biological replicates for each experimental condition. All these samples were sequenced in the same batch. 4SU: HeLa cells were treated with 4-thiouridine (4SU) for 16 h before photo-crosslinking. M3 = METTL3; M14 = METTL14. <sup>b</sup> Trimmed reads: The raw reads of PAR-CLIP were trimmed of 3' adaptors. <sup>c</sup> Alignment ratio = Mappable reads/Trimmed reads.

**Supplementary Table 11. m<sup>6</sup>A-seq data summary<sup>a</sup>**

Sample name	Total reads	Unmappable reads	Mappable reads	Mappable ratio <sup>b</sup>	Number of m <sup>6</sup> A peaks
C1-Input-1	24974813	10590363	14384450	57.60%	
C1-Input-2	33006218	15755676	17250542	52.26%	
C1-IP-1	41165316	10074178	31091138	75.53%	12069
C1-IP-2	23335490	5728242	17607248	75.45%	14546
M14-Input-1	30456486	11234147	19222339	63.11%	
M14-Input-2	29987090	9930717	20056373	66.88%	
M14-IP-1	17039757	3789398	13250359	77.76%	6994
M14-IP-2	15117152	4107915	11009237	72.83%	10994
WTAP-Input-1	12341257	4564380	7776877	63.02%	
WTAP-Input-2	10901667	4284656	6617011	60.70%	
WTAP-IP-1	35188369	8787004	26401365	75.03%	6834
WTAP-IP-2	29970954	7416268	22554686	75.26%	8319
C2-Input-1	39713949	6676648	33037301	83.19%	
C2-Input-2	33939392	5756895	28182497	83.04%	
C2-IP-1	53736718	5437323	48299395	89.88%	10994
C2-IP-2	52313345	5327449	46985896	89.82%	10543
M3-Input-1	39341532	8394091	30947441	78.66%	
M3-Input-2	39190870	8437406	30753464	78.47%	
M3-IP-1	46890761	4788203	42102558	89.79%	9410
M3-IP-2	45574392	4686727	40887665	89.72%	9389

<sup>a</sup> Two biological replicates for each experimental condition. C1 = control 1; C2 = control 2; M3 = METTL3; M14 = METTL14; IP = immunoprecipitation. The samples C1, M14, and WTAP were sequenced in the same batch, while C2 and M3 were run in the same batch. <sup>b</sup> Mappable ratio = Mappable reads/Total reads.

## Supplementary Note 1

### Protein sequence alignments of METTL3, IME4, METTL14, and METTL4

<b>METTL3</b>	MSDTWSSIQAHKKQLDSLRLRQRRRKQDSGHLDLRNPEAALSPTFRSDS	50
<b>IME4</b>	-----MINDKLVHFLIQNYDDILRAPLSGQL	26
<b>METTL14</b>	-----	
<b>METTL4</b>	-----MSVVHQLSAGWLLDHL	16
<b>METTL3</b>	PVPTAPTSGGPKPSTASAVPELATDPELEKKLLLHHILSDIALTLPTDAVSI	100
<b>IME4</b>	KDVYSLYISGGYDDEM <b>Q</b> KLRNDKDEVLQFEQFWNDLQDIIFATP-QSIQF	75
<b>METTL14</b>	-----	
<b>METTL4</b>	SFINKINYQLHQHHEPCCRKEFTTSVHFESI <b>Q</b> MDS-----VSSSGV	58
<b>METTL3</b>	CLAISTPDAPATQDGVESLLQKFAAQELIEVKRG <b>L</b> QDDAHPTLVTYADH	150
<b>IME4</b>	DQNLLVADRPEKIVYLD <b>V</b> FSLKILYNKFHAFYYTLKSSSSS-----CE	118
<b>METTL14</b>	-----MDSR <b>I</b> QEIRERQKLRRQLLAQQQLGAES-----A	28
<b>METTL4</b>	CAAFIASDSSTKPENDDGGNYEMFT <b>R</b> KFVFRPELFDVTKPY-----	99
<b>METTL3</b>	SKLSAMMGAVA <b>E</b> KKGPGEVAGTVTGQKRR <b>A</b> E <b>Q</b> DSTTVAAFASSLVSGLNS	200
<b>IME4</b>	EKVSSLTTKPEADSEKD <b>Q</b> LLGRLLG-----V <b>L</b> NWDVNVS <b>N</b> --QG	155
<b>METTL14</b>	DSIGAVLNSKDE <b>Q</b> REIAETR-----E	49
<b>METTL4</b>	-ITPAVHK <b>E</b> CQQSNE <b>K</b> EDLMN-----G	120
<b>METTL3</b>	SASEPAKEPAKKSRKHAASDV <b>D</b> LEIESLLNQQST <b>K</b> EQQS <b>K</b> KVS <b>Q</b> E <b>I</b> ELL	250
<b>IME4</b>	LPREQLSNRLQNLLREKPSS <b>F</b> QLAKERAKYTTEVIEY <b>I</b> PICSDYSHAS <b>LL</b>	205
<b>METTL14</b>	TCRASYDTSAPNAKRKY <b>L</b> DEGETDED <b>K</b> MEEY <b>K</b> DELEM <b>Q</b> QDEENLPYEEEI	99
<b>METTL4</b>	V <b>K</b> KEISISII <b>G</b> KKRKRCVVFNQ <b>G</b> ELDAMEYHT <b>K</b> IREL <b>L</b> LD <b>G</b> SLQL <b>Q</b> EGL	170
<b>METTL3</b>	NTTTAKEQS <b>I</b> VEKFRSRGRA <b>Q</b> V <b>Q</b> EFC <b>D</b> YGTKEEC <b>M</b> KAS <b>D</b> ADRPCRKLH <b>F</b> R	300
<b>IME4</b>	STS <b>V</b> YIVNN <b>K</b> IVS <b>L</b> QWS <b>K</b> ISAC <b>Q</b> ENHPG-- <b>L</b> IECIQS-----K <b>I</b> HFI	245
<b>METTL14</b>	YKDSSTFLKG <b>T</b> QSLNPHN-DYC <b>Q</b> H <b>F</b> VD <b>T</b> G-----	127
<b>METTL4</b>	KSGFLYPLFEK <b>Q</b> DKGS <b>K</b> PITLPLD <b>A</b> CSL <b>S</b> EL <b>C</b> MAK <b>H</b> LP-----SLN	212
<b>METTL3</b>	RI <b>I</b> INKHTDES <b>L</b> GDCSF <b>L</b> NTCF <b>H</b> MD <b>T</b> CKY <b>V</b> HY--E <b>I</b> DACMD <b>S</b> E <b>A</b> P---GSK	345
<b>IME4</b>	PNIKPQT <b>D</b> ISLGDCSYLD <b>T</b> CH <b>K</b> LNT <b>C</b> RY <b>I</b> HY <b>L</b> QY <b>I</b> P <b>S</b> CL <b>Q</b> ERAD <b>R</b> ET <b>A</b> SE	295
<b>METTL14</b>	---HRPQN <b>F</b> IRD <b>V</b> GLADR <b>F</b> EEY <b>P</b> KL <b>R</b> EL <b>I</b> R---LK <b>D</b> ELIA <b>K</b> SNT-----	165
<b>METTL4</b>	EMEHQ <b>T</b> QL <b>V</b> EE <b>D</b> TSV <b>T</b> EQDLFLRV <b>V</b> EN <b>N</b> SSFT <b>K</b> VIT <b>L</b> MG <b>Q</b> KY <b>LL</b> P---	258
<b>METTL3</b>	DHTPS <b>Q</b> ELALT <b>Q</b> SVGG <b>D</b> SS <b>AD</b> --RLFPP <b>Q</b> W <b>I</b> CC <b>D</b> IRY <b>L</b> D-V <b>S</b> ILG-KFA	390
<b>IME4</b>	NKR <b>I</b> RS <b>N</b> V <b>S</b> IP <b>F</b> YTLGNCS <b>A</b> HC <b>I</b> KK <b>AL</b> PA <b>Q</b> W <b>I</b> CD <b>V</b> R <b>K</b> FD-F <b>R</b> VLG-KFS	343
<b>METTL14</b>	-----PPMY <b>I</b> Q <b>A</b> DI <b>E</b> AFD-I <b>R</b> ELTP <b>K</b> FD	187
<b>METTL4</b>	-----PKSS <b>F</b> LLSD <b>I</b> SC <b>M</b> Q <b>P</b> LL <b>N</b> Y <b>R</b> KT <b>F</b> D	282

<b>METTL3</b>	VVMADPPWDIHME <sub>L</sub> PYGTLTD-----DEMRRILNIPVLQD-DGFLFLWVT-	433
<b>IME4</b>	VVIADPAWN <sub>I</sub> HMNLPYGT <sub>C</sub> ND-----IELLGLPLHEI <sub>L</sub> QD-EGIIIFLWVT-	386
<b>METTL14</b>	VILLEPPIEEYYRETGITANEKCWTWDDIMKLEIDEIAAPRSFIFLWC <sub>G</sub> S	237
<b>METTL4</b>	VIVIDPPWQNKS <sub>V</sub> KRSNRYSYLSP--LQIQQIPIP <sub>K</sub> LAA <sub>P</sub> NCLLV <sub>T</sub> WVTN	330
<b>METTL3</b>	-GRAMELGRECLN-LWGYERVDEIIWVKTNQLQR-II <sub>R</sub> TGRTGHWL <sub>N</sub> HGK	480
<b>IME4</b>	-GRAIELGKESLN-NWGYNVINEVSWIKTNQLGR-TIVTGR <sub>T</sub> GHWL <sub>N</sub> HSK	433
<b>METTL14</b>	-GEGLDLGRVCLR-KWGYRRCEDICWI <sub>K</sub> TNKNNPGKT <sub>K</sub> TLDPKAVFQRTK	285
<b>METTL4</b>	RQKH <sub>L</sub> RF <sub>I</sub> KEELYPSWSVEVVAEWHWVKITNSGEFVFPLDSPHKKPYEGL	380
<b>METTL3</b>	EHCLVGVKGNPQ-----GFNQGLDCDVIVAEVRSTS--HKPDEIYGMIE	522
<b>IME4</b>	EHLLVGLKG <sub>N</sub> PK-----WINKHIDVDLIVSMTRETS--RKPDELYGIAE	475
<b>METTL14</b>	EHCLMG <sub>I</sub> KGTVKRSTDGDFIHANV <sub>D</sub> IDLI <sub>I</sub> TEEPEIGNIEKPVEIFHI <sub>I</sub> E	335
<b>METTL4</b>	ILGRVQEKTALPLRNADVNLPIPDHKLIVSV <sub>P</sub> CTLH--SHKPPLAEVLK	428
<b>METTL3</b>	RLS-PGT <sub>R</sub> KIELFGRPHNVQP <sub>N</sub> WITLGNQLDG <sub>I</sub> HLLDPDV <sub>V</sub> ARFKQ <sub>R</sub> YPD	571
<b>IME4</b>	RLAGTHARKLEIFGRDHNT <sub>R</sub> PGWFTIGNQLTGNCIYEMDVERKYQE <sub>F</sub> MKS	525
<b>METTL14</b>	HFC-LGRRRLH <sub>I</sub> LFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSY	384
<b>METTL4</b>	DYIKPDGEYI <sub>E</sub> LFAR--NLQPGWTSGNEV <sub>L</sub> KFQHVDYFIAVESGS----	472
<b>METTL3</b>	GIIS-----KPKNL-----	580
<b>IME4</b>	KTGTSHTGT <sub>K</sub> KIDKKQPSKLQQQHQQQY <sub>W</sub> NNMDMGS <sub>G</sub> KYYAEAKQNPM <sub>N</sub> Q	575
<b>METTL14</b>	LTGCT----EEIERLRPKSPPP <sub>K</sub> S <sub>K</sub> SDRGGGAPRG <sub>GG</sub> RG <sub>G</sub> TSAGRGRERN	430
<b>METTL4</b>	-----	
<b>METTL3</b>	-----	
<b>IME4</b>	KHTPFESKQQQKQQFQT <sub>L</sub> NNLYFAQ-	600
<b>METTL14</b>	RSNFRGERGGFRGGRGGAHRGGFPPR	456
<b>METTL4</b>	-----	

## Supplementary Note 2

### Protein sequence alignments of METTL3 across human, animal, plant, and yeast

<b>Homo sapiens</b>	MSDTWSSIQAHKKQLDSL RERLQRRRK----QDSGHLDLRN---PE---AALS----PT
<b>Canis lupus familiaris</b>	MSDTWSSIQAHKKQLDSL RERLQRRRK----QDSGHLDLRN---PE---AALS----PN
<b>Macaca mulatta</b>	MSDTWSSIQAHKKQLDSL RERLQRRRK----QDSGHLDLRN---PE---AALS----PT
<b>Rattus norvegicus</b>	MSDTWSSIQAHKKQLDSL RERLQRRRK----QDSGHLDLRN---PE---AALS----PT
<b>Mus musculus</b>	MSDTWSSIQAHKKQLDSL RERLQRRRK----QDSGHLDLRN---PE---AALS----PT
<b>Bos Taurus</b>	MSDTWSSIQAHKKQLDSL RERLQRRRK----QDSGHLDLRN---PE---AALS----PT
<b>Danio rerio</b>	MSDTWSHIQAHKKQLDSL RERLQRRRK----DETQLGTEV----G----SVES----GS
<b>Aedes aegypti</b>	-MSSWEIHALKAKRNTL RERLEKRKK----ERQDLLGGSS--PG----PSVV----GL
<b>Drosophila melanogaster</b>	-MADAWDIKS LKT KRNTL REKLEKRKK----ERIEILSDIQ---ED---LTNP-----
<b>Arabidopsis thaliana</b>	-METESD-DATITVVVKDMRVRLENRIRTQHDAHLDLLSSLQSIVPDIVPSLDLSLKLISS
<b>Saccharomyces cerevisiae</b>	-----

<b>Homo sapiens</b>	FR-----SDSPVP--TAPTSGGPKPSTA-SAVPEL-----ATDPEL-----
<b>Canis lupus familiaris</b>	FR-----SDSPVP--TVPTSGGPKPSTA-SAVPEL-----ATDPEL-----
<b>Macaca mulatta</b>	FR-----SDSPVP--TAPTSGGPKPSTA-SAVPEL-----ATDPEL-----
<b>Rattus norvegicus</b>	FR-----SDSPVP--TAPTSGGPKPSTT-SIVPEL-----ATDPEL-----
<b>Mus musculus</b>	FR-----SDSPVP--TAPTSGGPKPSTT-SVAPEL-----ATDPEL-----
<b>Bos Taurus</b>	FR-----SDSPVP--AAPTSGGPKPSTA-SAVPEL-----ATDPEL-----
<b>Danio rerio</b>	AR-----SDSPGP--AIQ-----SPP-QVEVEH-----PPDPEL-----
<b>Aedes aegypti</b>	IK-----TEAGVS--SVDDK--SKILLT-AIKLEQ-----DIDAEV-----
<b>Drosophila melanogaster</b>	-----KKELV-----EADLEV-----
<b>Arabidopsis thaliana</b>	FTNRPFVATPPLPEPKVEKKHHPIVKLGTQLQQLHGHD SKMLVDSNQRDAEADGSSGSP
<b>Saccharomyces cerevisiae</b>	-----

<b>Homo sapiens</b>	-----EKKLLHHLS DLALTLP TD AVSICLAISTPD--APATQDGVES-----L
<b>Canis lupus familiaris</b>	-----EKKLLHHLS DLALTLP TD AVSIRLAISTPD--APATQDGVES-----L
<b>Macaca mulatta</b>	-----EKKLLHHLS DLALTLP TD AVSICLAISTPD--APATQDGVES-----L
<b>Rattus norvegicus</b>	-----EKKLLHHLS DLALTLP TD AVSIRLAISTPD--APATQDGVES-----L
<b>Mus musculus</b>	-----EKKLLHHLS DLALTLP TD AVSIRLAISTPD--APATQDGVES-----L
<b>Bos Taurus</b>	-----EKKLLHHLS DLSL TLPTD AVSIRLAISTPD--APATQDGVES-----L
<b>Danio rerio</b>	-----EKRLLGYLSEL SLSL PTD SLT ITNQLNTSE--SPVSHSCIQS-----L
<b>Aedes aegypti</b>	-----EKT LVKVLADR SLILPSNSMQIA QRVEKIIQ-RSTTNESILY-----C
<b>Drosophila melanogaster</b>	-----QKEVILQALSSCSL ALPIV STQVVEKI---A---GSSLEMVN F-----I
<b>Arabidopsis thaliana</b>	MALVRAMVAECLL QRVFSPTDSSTVLRKLENDQN ARPAEKAALRDL GGECPILAVETA
<b>Saccharomyces cerevisiae</b>	--MINDKLVHFLI QNY-----

<b>Homo sapiens</b>	LQKFA-AQELIEVKRGLLQDDAHPTLVTYADHSK-LSAMMGAV---AEKKGPG--EVAG-
<b>Canis lupus familiaris</b>	LQKFA-AQELIEVKRGLLQDDAHPTLVTYADHSK-LSAMMGAV---AEKKGPG--EVAG-
<b>Macaca mulatta</b>	LQKFA-AQELIEVKRGLLQDDAHPTLVTYADHSK-LSAMMGAV---AEKKGPG--EVAG-

<b>Rattus norvegicus</b>	LQKFA-AQELIEVKRGLLQDDAHPTLVTYADHSK-LSAMMGAV---AEKKGLG--EAAG-
<b>Mus musculus</b>	LQKFA-AQELIEVKRGLLQDDAHPTLVTYADHSK-LSAMMGAV---AEKKGLG--EVAG-
<b>Bos Taurus</b>	LQKFA-AQELIEVKRSLLQDDAHPTLVTYADHSK-LSAMMGAV---AEKKGP--EVAG-
<b>Danio rerio</b>	LLKFS-AQELIEVRQPSITSSSSTLVTSDHTK-LWAMIGSA---GQSQRTAVKRKAD-
<b>Aedes aegypti</b>	LQKLS-GQNLVNIKEVSIGGTVGYEVIS-AEYGK-IQSLHDNLLN-QSEREAIKRKAI-
<b>Drosophila melanogaster</b>	LGKLA-NQGAIVIRNVTIGTEAGCEIIS-VQPKE-LKEILEDTNDTCQQKEEEAKRKE-
<b>Arabidopsis thaliana</b>	LKSMAEENGSVLEEFEVSGKPRIMVLA-IDTRLLKELPESFQGNNESNRV--VETPNS
<b>Saccharomyces cerevisiae</b>	-----DDILRAPLSGQLKDVSILYI-----SGGYDDEMQ-----
<b>Homo sapiens</b>	----TVTGQKRRAEQDSTTV-----AAFASSL-----VSGLNSS-----
<b>Canis lupus familiaris</b>	----TITGQKRRAEQDSTTV-----AAFASSL-----ASGLASS-----
<b>Macaca mulatta</b>	----TVTGQKRRAEQDSTTV-----AAFASSL-----ASGLSSS-----
<b>Rattus norvegicus</b>	----TITGQKRRAEQDLTTV-----ATFTSSL-----ASGLGSS-----
<b>Mus musculus</b>	----TIAGQKRRAEQDLTTV-----TTFASSL-----ASGLASS-----
<b>Bos Taurus</b>	----TIAGQKRRAEQDLTTA-----AAFTSSL-----ASGLASS-----
<b>Danio rerio</b>	----DITHQKRAPGSSPSIQAPPSPPRKSSVSIATAS-----ISQLTASSG-----
<b>Aedes aegypti</b>	-----KEEMDSDSKMAR-----LG-----
<b>Drosophila melanogaster</b>	-----VDDVDQ-----PQ-----
<b>Arabidopsis thaliana</b>	IENATVSGGGFGVSGSGNFPRPEMWGGDPNMGFRPMNNAPRGMQMMGMHHPMGIMGRPPP
<b>Saccharomyces cerevisiae</b>	-----KLRNDKDEVLQFEQFWNDLQDIIFATPQSI-----Q-----FDQNLLVADRP-----
<b>Homo sapiens</b>	ASEPAKEPA-----KKSRKHAASDV DLEIESLLNQQSTKEQQSKKV-----
<b>Canis lupus familiaris</b>	ASEA AKEPT-----KKSRKHAASDV DLEIESLLNQQSTKEQQSKKV-----
<b>Macaca mulatta</b>	ASEPAKEPA-----KKSRKHAASDV DLEIESLLNQQSTKEQQSKKV-----
<b>Rattus norvegicus</b>	TSEPAKEPA-----KKSRKHAASDV DLEIESLLNQQSTKEQQSKKV-----
<b>Mus musculus</b>	ASEPAKEPA-----KKSRKHAASDV DLEIESLLNQQSTKEQQSKKV-----
<b>Bos Taurus</b>	ASEVAKEPT-----KKSRKHAASDV DLEIESLLNQQSTKEQQSKKV-----
<b>Danio rerio</b>	GGGGGADKK-----GRSNKVQASHLDMEIESLLSQQSTKEQQSKKV-----
<b>Aedes aegypti</b>	GGLVKEESL-----KKPGDSSTLSSTS DIMSLLSLPSTREKQSKQV-----
<b>Drosophila melanogaster</b>	EKTIKLEST-----VARKEESTSLDAPDDIMML SMPSTREKQSKQV-----
<b>Arabidopsis thaliana</b>	FPLPLPLPVP-----SNQKLRSEEDILKDV EALLSKKS FKEKQQSRT-----
<b>Saccharomyces cerevisiae</b>	EKIVYLDVFSLKILYNKFHAFYYTLKSSSSC EEVK VSSLTTKPEADSEKDQLLGRILLGV-----
<b>Homo sapiens</b>	-----SQEILELLNTTTAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Canis lupus familiaris</b>	-----SQEILELLNTTTAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Macaca mulatta</b>	-----G-----VIQEILELLNTTTAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Rattus norvegicus</b>	-----SQEILELLNTTTAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Mus musculus</b>	-----SQEILELLNTTTAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Bos Taurus</b>	-----SQEILELLNTTTAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Danio rerio</b>	-----SQEILELLNTSSAKEQSIVEKFRSRGRAQVQEFC DYG-----TKEE
<b>Aedes aegypti</b>	-----GEEILELLTKPTAKER S LAEKFKS QGGAQVMEFC PHG-----TRIE
<b>Drosophila melanogaster</b>	-----GEEILELLTKPTAKER S VA EKFKSHGGAQVMEFC SHG-----TKVE
<b>Arabidopsis thaliana</b>	-----GEELLDL IHRPTAKEAA TAAKFKSKGGSQVKYYCRYL-----TKED
<b>Saccharomyces cerevisiae</b>	NWDVN VSNQGLPREQLSNRLQNLREK PSSF--QLAKERAKYTTEVIEYIPICSDYSHAS

Homo sapiens	CMKASD-----
Canis lupus familiaris	CMKASD-----
Macaca mulatta	CMKASD-----
Rattus norvegicus	CMKASD-----
Mus musculus	CMKASD-----
Bos Taurus	CMKASD-----
Danio rerio	CVQSGD-----
Aedes aegypti	CLRSLEAANDALLKSEDD DD VIISDDN-----NGFEIVEIK-----
Drosophila melanogaster	CLKAQQATAEMAACKKKQERRDEKELRPDVDAGENVTGKVPKTESAAEDGEIIAEVINNCE
Arabidopsis thaliana	CRLQSG-----
Saccharomyces cerevisiae	LLSTSV-----YI-----VN-NKIVSLQWSKI

Homo sapiens	-----ADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Canis lupus familiaris	-----ADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Macaca mulatta	-----ADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Rattus norvegicus	-----ADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Mus musculus	-----ADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Bos Taurus	-----ADRPCRKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Danio rerio	-----TPQPCTKLHFRRIINKHTDESLGDCSFLNTCFHMDTCKYVHYEI
Aedes aegypti	-----KEKDDD KIKFQCNKLHFKKIIQSHTDESLGDCSFLNTCFHMDTCKYVHYEV
Drosophila melanogaster	AESQESTDGS DTCSETTDKCTKLHFKKIIQAHTDESLGDCSFLNTCFHMDTCKYVHYEV
Arabidopsis thaliana	-----SHIACNKRFRRLIAHSHTDVS LGDCSFLDTCRHMKTCKYVHYEL
Saccharomyces cerevisiae	SACQ-----ENHPGLIECIQS KIH FIPNIKPQTDISLGDCSYLDTCHKLN TCRYIH YLQ

Homo sapiens	DACMDSEAPGSKDHTPSQ-----ELALTQSVGGDS---SADRLFPPQWICCDIRYLDVSI
Canis lupus familiaris	DACMDSEAPGSKDHTPSQ-----ELALTQSVGGDS---NADRLFPPQWICCDIRYLDVSI
Macaca mulatta	DACMDSEAPGSKDHTPSQ-----ELALTQSVGGDS---SADRLFPPQWICCDIRYLDVSI
Rattus norvegicus	DACVDSESPGSKEHMPSQ-----ELALTQSVGGDS---SADRLFPPQWICCDIRYLDVSI
Mus musculus	DACVDSESPGSKEHMPSQ-----ELALTQSVGGDS---SADRLFPPQWICCDIRYLDVSI
Bos Taurus	DACMDSEAPGSKDHTPSQ-----ELALTQSVGGDS---SADRLFPPQWICCDIRYLDVSI
Danio rerio	DSPPEAEGDALGPQAGAA-----ELGLHS-TVGDS---NVGKLFPSQWICCDIRYLDVSI
Aedes aegypti	DTYVGQNTGS KFE-----GEAS---KRTID---PCATLYPPQWIQC DLRFLDMTV
Drosophila melanogaster	DTLP HINTN KPTDVKT-----KLSL---KRSVD---SSCTLYPPQWIQC DLRFLDMTV
Arabidopsis thaliana	DMADAMMAGPDKALK-----PLRADYC---SEALGEAQWINCDIRSFRMDI
Saccharomyces cerevisiae	YIPSCLQERADRETASENKRIRSNVSIPFYTLGNCSAHC IKKALPAQWIRC DVRKFD FRV

Homo sapiens	LGKFAVV MADPPWDIH MELPYGTLT DDEM RRLN I PVL QDDGFLFLWVTG RAME LGRE CLN
Canis lupus familiaris	LGKFAVV MADPPWDIH MELPYGTLT DDEM RRLN I PVL QDDGFLFLWVTG RAME LGRE CLN
Macaca mulatta	LGKFAVV MADPPWDIH MELPYGTLT DDEM RRLN I PVL QDDGFLFLWVTG RAME LGRE CLN
Rattus norvegicus	LGKFAVV MADPPWDIH MELPYGTLT DDEM RRLN I PVL QDDGFLFLWVTG RAME LGRE CLN
Mus musculus	LGKFAVV MADPPWDIH MELPYGTLT DDEM RRLN I PVL QDDGFLFLWVTG RAME LGRE CLN
Bos Taurus	LGKFAVV MADPPWDIH MELPYGTLT DDEM RRLN I PVL QDDGFLFLWVTG RAME LGRE CLN

<b>Danio rerio</b>	LGKFAVVMADPPWDIHME <sub>L</sub> PYGTLTDEMRKLNIPILQDDGFLFLWVTGRAMELGRECLS
<b>Aedes aegypti</b>	LGKFAVVMADPPWDIHME <sub>L</sub> PYGTMSDDEM <sub>R</sub> QIGVPALQDDGLIFLWVTGRAMELGRECLK
<b>Drosophila melanogaster</b>	LGKFAVVMADPPWDIHME <sub>L</sub> PYGTMSDDEM <sub>R</sub> ALGVPALQDDGLIFLWVTGRAMELGRCCLK
<b>Arabidopsis thaliana</b>	LGTFGVVMADPPWDIHME <sub>L</sub> PYGT <sub>M</sub> ADDEM <sub>T</sub> LN <sub>V</sub> PSLQT <sub>D</sub> GLIFLWVTGRAMELGRECLE
<b>Saccharomyces cerevisiae</b>	LGKF <sub>S</sub> VVIADPAWN <sub>I</sub> HMNLPYGT <sub>C</sub> N <sub>D</sub> IELLGLPLHEI <sub>L</sub> QDEGI <sub>I</sub> FLWVTGR <sub>A</sub> IELGKESLN
<b>Homo sapiens</b>	LWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVA
<b>Canis lupus familiaris</b>	LWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVA
<b>Macaca mulatta</b>	LWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVA
<b>Rattus norvegicus</b>	LWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVA
<b>Mus musculus</b>	LWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVA
<b>Bos Taurus</b>	LWGYERVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNQGLDCDVIVA
<b>Danio rerio</b>	LWGYDRVDEIIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVGVKGNPQGFNRGLDCDVIVA
<b>Aedes aegypti</b>	LWGYERVDELIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVG <sub>M</sub> KGNPPNLNRGLDCDVIVA
<b>Drosophila melanogaster</b>	LWGYERVDELIWVKTNQLQRIIRTGRTGHWLNHGKEHCLVG <sub>M</sub> KGNPTNLNRGLDCDVIVA
<b>Arabidopsis thaliana</b>	LWGYKRVEEI <sub>I</sub> WVKTNQLQRIIRTGRTGHWL <sub>N</sub> SKEHCLVG <sub>I</sub> KGNPE-VNRNIDTDVIVA
<b>Saccharomyces cerevisiae</b>	NGYNVINEVSWIKTNQLGRTIVT <sub>G</sub> RTGHWL <sub>N</sub> SKEHLLVGLKGNPKWINKHIDVDLIVS
<b>Homo sapiens</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPDVVAR
<b>Canis lupus familiaris</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPDVVAR
<b>Macaca mulatta</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPDVVAR
<b>Rattus norvegicus</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPDVVAR
<b>Mus musculus</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPDVVAR
<b>Bos Taurus</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPDVVAR
<b>Danio rerio</b>	EVRSTSHKPDEIYGMIERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> HLLDPEVVAR
<b>Aedes aegypti</b>	EVRATSHKPDEIYG <sub>I</sub> IERLSP-GTRKIELFGRPHNVQPNWITLGNQLDG <sub>I</sub> RLVDP <sub>E</sub> LISS
<b>Drosophila melanogaster</b>	EVRATSHKPDEIYG <sub>I</sub> IERLSP-GTRKIELFGRPHNIQPNWITLGNQLDG <sub>I</sub> RLVDP <sub>E</sub> LITQ
<b>Arabidopsis thaliana</b>	EVRET <sub>S</sub> RKPDEMYAMLERIMP-RARKLELFARMHN <sub>A</sub> HAGWL <sub>S</sub> GNQLNGVRLINEGLRAR
<b>Saccharomyces cerevisiae</b>	MTRETSRKPD <sub>E</sub> LYGIAERLAGTHARKLEIFGRDHNT <sub>P</sub> GWFTIGNQLTGNCIYEMDVERK
<b>Homo sapiens</b>	FKQRYPDGIIS-----KPKNL-----
<b>Canis lupus familiaris</b>	FKQRYPDGIIS-----KPKNL-----
<b>Macaca mulatta</b>	FKQRYPDGIIS-----KPKNL-----
<b>Rattus norvegicus</b>	FKHRYPDGVIS-----KPKNL-----
<b>Mus musculus</b>	FKQRYPDGIIS-----KPKNL-----
<b>Bos Taurus</b>	FKQRYPDGIIS-----KPKNL-----
<b>Danio rerio</b>	FKKRYPDGVIS-----KPKNM-----
<b>Aedes aegypti</b>	FQKRYPDGNCM-----TPGKNP-----
<b>Drosophila melanogaster</b>	FQKRYPDGNCM-----SPASANAASIN-----GIQK-----
<b>Arabidopsis thaliana</b>	FKASYPEIDVQ-----PPSPPRASAME-----TDNEPMAIDS <sub>I</sub>
<b>Saccharomyces cerevisiae</b>	YQEFMKSKTGT <sub>S</sub> HGT <sub>K</sub> KIDKKQPSKLQQQHQQQY <sub>W</sub> NNMDMSGKYYAEAKQNPMNQKHT
<b>Homo sapiens</b>	-----
<b>Canis lupus familiaris</b>	-----

*Macaca mulatta* -----  
*Rattus norvegicus* -----  
*Mus musculus* -----  
*Bos Taurus* -----  
*Danio rerio* -----  
*Aedes aegypti* -----  
*Drosophila melanogaster* -----  
*Arabidopsis thaliana* TA-----  
*Saccharomyces cerevisiae* PFESKQQQKQQFQTLNLYFAQ

### Supplementary Note 3

#### Protein sequence alignments of METTL14 across human and animal

<b>Homo sapiens</b>	-----
<b>Pan troglodytes</b>	-----
<b>Macaca mulatta</b>	-----
<b>Canis lupus familiaris</b>	MWSAGFQTNNHHQSQS <span style="color: red;">LVVTILRGRRFYNSQSTNEKPGAHGGLIESLLAKDTPPGKASPG</span>
<b>Bos taurus</b>	-----
<b>Mus musculus</b>	-----
<b>Rattus norvegicus</b>	-----
<b>Gallus gallus</b>	-----
<b>Danio rerio</b>	-----
<b>Drosophila melanogaster</b>	-----
<b>Anopheles gambiae PEST</b>	-----

<b>Homo sapiens</b>	-----MDSRLQEIRERQKLRRQLLAQQLGAESADSIGAVLNSKDEQ
<b>Pan troglodytes</b>	-----MDSRLQEIRERQKLRRQLLAQQLGAESADSIGAVLNSKDEQ
<b>Macaca mulatta</b>	-----MDSRLQEIRERQKLRRQLLAQQLGAESADSIGAVLNSKDEQ
<b>Canis lupus familiaris</b>	FKPDAPVQLFGAVGNRCWDRDTPFQEIRERQKLGGQLLAQQLGAESADSIGAVLNSKDEQ
<b>Bos taurus</b>	-----MDSRLQEIRERQKLRRQLLAQQLGAESADSIGAVLNSKDEQ
<b>Mus musculus</b>	-----MDSRLQEIRERQKLRRQLLAQQLGAESADSIGAVLNSKDEQ
<b>Rattus norvegicus</b>	-----MSLGAESADSIGAVLNSKDEQ
<b>Gallus gallus</b>	-----MNSRLQEIRERQKLRRQLLAQQLGAENADSIGAVLNSKDDQ
<b>Danio rerio</b>	-----MNSRLQEIRERQKLRRQLLAQQLGAESPDSIGAVLNSKDEQ
<b>Drosophila melanogaster</b>	-----MSDVLKSSQERSRKRLLLAQTGLLSSVDDLKKALGNAEDI
<b>Anopheles gambiae PEST</b>	-----MSDVIKSREKSQKRKMLLAQTFGVSCVEDLKHVLTGAEDS

<b>Homo sapiens</b>	REIAETRETCRASYDTSAPNAKRKYLDDEGETDEDKMEYKDELEMQQDEENLPYEEEIYK
<b>Pan troglodytes</b>	REIAETRETCRASYDTSAPNAKRKYLDDEGETDEDKMEYKDELEMQQDEENLPYEEEIYK
<b>Macaca mulatta</b>	REIAETRETCRASYDTSAPNTKRKYLDDEGETDEDKMEYKDELEMQQEEENLPYEEEIYK
<b>Canis lupus familiaris</b>	REIAETRETCRASYDTSAPNAKRKYQDEGETDEDKMEYKDELEMQQEEENLPYEEEIYK
<b>Bos taurus</b>	REIAETRETCRASYDTSAPNAKRKYQDEGETDEDKMEYKDELEMQQEEENLPYEEEIYK
<b>Mus musculus</b>	REIAETRETCRASYDTSAPNSKRKCLDEGETDEDKVEEYKDELEMQQEEENLPYEEEIYK
<b>Rattus norvegicus</b>	REIAETRETCRASYDTSAPNSKRKCLDEGETDEDKVEEYKDELEMQQEEENLPYEEEIYK
<b>Gallus gallus</b>	REIAETRETCRASYDTSAPNAKRKYPDEGEADEEEIEEYKDEVELQQDEENLPYEEEIYK
<b>Danio rerio</b>	KEIEETRETCRASFDISVPGAKRKCLNEGEDPEEDVEEQKEDVEPQHQEESGPYE-EVYK
<b>Drosophila melanogaster</b>	NSSR-----QLNSGGQREE-----EDGGASSSKTPNEIIYR
<b>Anopheles gambiae PEST</b>	PIKS-----Q-----RYED-----EEASTSKTVQTAEGLVYR

<b>Homo sapiens</b>	DSSTFLKGTSLSNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<b>Pan troglodytes</b>	DSSTFLKGTSLSNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<b>Macaca mulatta</b>	DSSTFLKGTSLSNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA

<i>Canis lupus familiaris</i>	DSSTFLKGTQSLNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<i>Bos taurus</i>	DSSTFLKGTQSLNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<i>Mus musculus</i>	DSSTFLKGTQSLNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<i>Rattus norvegicus</i>	DSSTFLKGTQSLNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<i>Gallus gallus</i>	DSSTFLKGTQSLNPHNDYCQHFVDTGHRPQN FIRDVGLADRFEYPKLRELIRLKDELIS
<i>Danio rerio</i>	DSSTFLKGTQSLNPHNDYCQHFVDTGHRPQN FIRDGLADRFEYPKQRELIRLKDELIS
<i>Drosophila melanogaster</i>	DSSTFLKGTQSSNPHNDYCQHFVDTGQRPQN FIRDVGLADRFEYPKLRELIKLKDQI
<i>Anopheles gambiae</i> PEST	DSSTFLKGTQSSNPHNDYCQHFVDTGQRPQN FIRDVGLADRFEYPKLRELIRLKDELIA
<i>Homo sapiens</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Pan troglodytes</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Macaca mulatta</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Canis lupus familiaris</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Bos taurus</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Mus musculus</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Rattus norvegicus</i>	KSNTPPMYLQADIEAFDIRELTPKFDVILLEPPL EYYRETGIA----ANEKCWTWDDI
<i>Gallus gallus</i>	KSNTPPMYLQADLEAFDIRELKSKFDVILLEPPL EYYRETGIT----ANEKCWTWDDI
<i>Danio rerio</i>	ATNTPPMYLQADPDTFDLRELKCKFDVILIEPPL EYYRESGII----ANERFWNWDDI
<i>Drosophila melanogaster</i>	DTASAPMYLKADLKSLDVKTLGAKFDVILIEPPL EYARAAPSATVGGA PRVFWNWDDI
<i>Anopheles gambiae</i> PEST	ETATPPMYLRADLKTFDLKNLGTKFDVILIEPPL EYARGAAV--AAGAPRNFWSWDEI
<i>Homo sapiens</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Pan troglodytes</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Macaca mulatta</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Canis lupus familiaris</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Bos taurus</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Mus musculus</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Rattus norvegicus</i>	MKLEIDEIAAPRSFIFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Gallus gallus</i>	MKLEIEEIAAPRSFVFLWCGSGEGLDLGRVCLRKWGYRRCEDICWI KTNKNNGKTKTLD
<i>Danio rerio</i>	MKLNIEEISSIRS FVFLWCGSGEGLDLGRMCLRKWGFRRCEDICWI KTNKNNGKTKTLD
<i>Drosophila melanogaster</i>	LNLDVGEIAAHRS FVFLWCGSSEGLDMGRNCLKKWGFRRCEDICWIRTNINKPGHSQLE
<i>Anopheles gambiae</i> PEST	LALDIGEVAAHRS FVFLWCGSSEGLDMGRNCLRKWGFRRCEDICWIRTNIDSPGHSKILE
<i>Homo sapiens</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Pan troglodytes</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Macaca mulatta</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Canis lupus familiaris</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Bos taurus</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Mus musculus</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Rattus norvegicus</i>	PKAVFQRTKEHCLMGIKGTVKRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Gallus gallus</i>	PKAVFQRTKEHCLMGIKGTVRRSTDGDFIHANVDIDLITTEEPEIGNIEKPVEIFHIEH
<i>Danio rerio</i>	PKAVFQRTKEHCLMGIKGTVRRSTDGDFIHANVDIDLITTEEPEMGNI EKPVEIFHIEH
<i>Drosophila melanogaster</i>	PKAVFQRTKEHCLMGIKGTVRRSTDGDFIHANVDIDLISEEEFGSFEKPIEIFHIEH
<i>Anopheles gambiae</i> PEST	PKAVFQRTKEHCLMGIKGTVRRSTDGDFIHANVDIDLISEEAEFGSLEKPIEIFHIEH

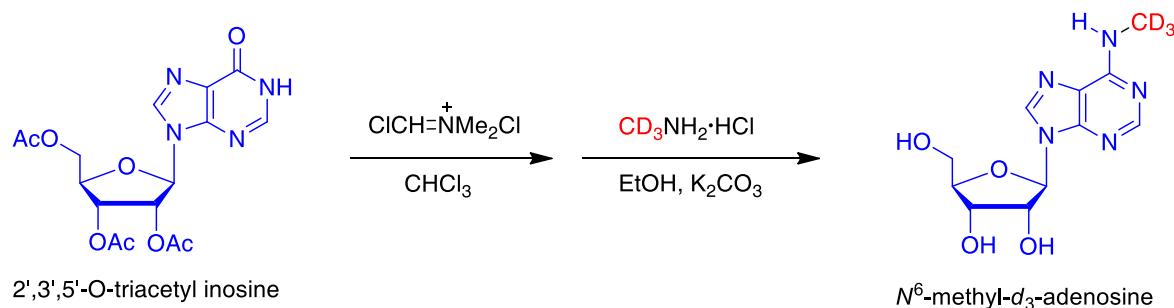
<b>Homo sapiens</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Pan troglodytes</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Macaca mulatta</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Canis lupus familiaris</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Bos taurus</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Mus musculus</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Rattus norvegicus</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNYNAETYASYFSAPNSYLTGCTEEIERLR
<b>Gallus gallus</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNFNAETYSSYFTAPNSHTGCTEEIERLR
<b>Danio rerio</b>	FCLGRRRLHIFGRDSTIRPGWLTVGPTLTNSNFNIEVYSTHFSEPNSYLSGCTEEIERLR
<b>Drosophila melanogaster</b>	FCLGRRRLHIFGRDSSIRPGWLTVGPELTNSNFNSELYQTYFAEA--PATGCTSRIELLR
<b>Anopheles gambiae PEST</b>	FCLGRRRLHIFGRDSTIRPGWVTIGPELTNSNFNSELYASSFEEN--PTTGCTERIEALR

<b>Homo sapiens</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGAHRGGF
<b>Pan troglodytes</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGAHRGGF
<b>Macaca mulatta</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGAHRGGF
<b>Canis lupus familiaris</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGAHRGGF
<b>Bos taurus</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGAHRGGF
<b>Mus musculus</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGTHRGGF
<b>Rattus norvegicus</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGR---GRERNRSNFRGERGGFRGGRRGGTHRGGF
<b>Gallus gallus</b>	PKSPPP <span style="color:red">KS</span> KSDRGGAPRGGRGGTSAGRGERGRERNRTNFRGERGGFRGGRRGGTHRGGF
<b>Danio rerio</b>	PKSPPP <span style="color:red">KS</span> MAERGGGAPRG-GRGGPAAGRGDRGRERNRPNFRGDRGGFRGRG-GPH-RGF
<b>Drosophila melanogaster</b>	PKSPPP <span style="color:red">NS</span> KVLGRGRGFPRGRGRPR-----
<b>Anopheles gambiae PEST</b>	PKSPPANG <span style="color:red">KV</span> LGRGRGFARGLRGRSRV-----

<b>Homo sapiens</b>	PPR
<b>Pan troglodytes</b>	PPR
<b>Macaca mulatta</b>	PPR
<b>Canis lupus familiaris</b>	PPR
<b>Bos taurus</b>	PPR
<b>Mus musculus</b>	TPR
<b>Rattus norvegicus</b>	TPR
<b>Gallus gallus</b>	PTR
<b>Danio rerio</b>	PPR
<b>Drosophila melanogaster</b>	---
<b>Anopheles gambiae PEST</b>	---

#### Supplementary Note 4

**Synthesis of *N*<sup>6</sup>-methyl-*d*<sub>3</sub>-adenosine (*d*<sub>3</sub>-m<sup>6</sup>A) free nucleoside.** *N*<sup>6</sup>-Methyl-*d*<sub>3</sub>-adenosine free nucleoside was synthesized by using methyl-*d*<sub>3</sub>-amine hydrogen chloride with potassium carbonate in ethanol. The structure of *N*<sup>6</sup>-methyl-*d*<sub>3</sub>-adenosine was confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR and Mass spectrometry (MS). Its <sup>1</sup>H NMR spectrum is similar to that of *N*<sup>6</sup>-methyladenosine except the resonance peak at 2.95 ppm (3H) disappears due to the replacement of CH<sub>3</sub> by CD<sub>3</sub>. Consistently, its MS also shows the molecular weight greater than that of *N*<sup>6</sup>-methyladenosine by 3.



**Synthetic procedure.** To a 100 mL flask was added 2',3',5'-*O*-triacetyl inosine (415 mg, 1.05 mmol) and anhydrous CHCl<sub>3</sub> (15 mL) to give a clear solution. Then (chloromethylene)dimethyliminium chloride (270 mg, 2.1 mmol, 2.0 equiv.) was added and the mixture was stirred and heated to reflux for 2 hours. After cooling down to room temperature, saturated NaHCO<sub>3</sub> was added slowly followed by CH<sub>2</sub>Cl<sub>2</sub> and water. The organic layer was separated, washed with brine. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure to give yellow oil. To this oil were added ethanol (10 mL), methyl-*d*<sub>3</sub>-amine hydrogen chloride (500 mg, 7.09 mmol) followed by K<sub>2</sub>CO<sub>3</sub> (968 mg) to give a cloudy solution. Water was added gradually until a clear solution was formed. After the mixture was stirred at room temperature overnight, all the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography eluting with 6–8% MeOH in CH<sub>2</sub>Cl<sub>2</sub> to afford product **2** (245 mg, 82%) as a white foam. <sup>1</sup>H NMR (500 MHz) (DMSO-*d*<sub>6</sub>) δ: 8.35 (s, 1H), 8.23 (br., 1H), 7.82 (br., 1H), 5.89 (m, 1H), 5.45 (m, 2H), 5.21 (m, 1H), 4.63 (m, 1H), 4.15 (m, 1H), 3.96 (m, 1H), 3.69 (m, 1H), 3.54 (m, 1H). <sup>13</sup>C NMR (125.8 MHz) (DMSO-*d*<sub>6</sub>) δ: 156.54, 153.87, 149.46, 141.10, 121.34, 89.34, 87.35, 74.90, 72.11, 63.12, 28.41. MS calculated for C<sub>11</sub>H<sub>12</sub>D<sub>3</sub>N<sub>5</sub>O<sub>4</sub>, [MH<sup>+</sup>] 285 (calcd.), 285 (found).